# Potato Tuberization in Hydroponics

By

**Simon Shengyuan Yang**

**GradDip Agr Sci (Hons) University of Tasmania**

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# ABSTRACT

The project investigated potato tuber initiation in hydroponics and was initiated by Sunrise Seed Potatoes Pty Ltd, a company specialising in potato minituber production in Tasmania, Australia. The initial focus of the project was to describe in detail the rate and timing of stolon and tuber development in nutrient film technique (NFT) hydroponics. A developmental scale consisting of five stages, each based on characteristic morphological changes, was developed to enable quantitative evaluation of the effects of experimental treatments on tuberization. Nutrient uptake rate at plant development stages was studied in different seasons. Uptake rate of water and nutrient elements generally displayed a decline from early to late stage regardless of season. Uptake rate of $H^+$ displayed significant variation between seasons and did not follow the characteristic decline.

A novel system for frequent and accurate non-destructive assessment of stolon and tuber growth using webcams and image analysis software was developed and validated. Stolon and tubers displayed a diurnal growth pattern with rapid elongation commencing mid to late afternoon and ceasing early morning, followed by a period of slower growth rate often leading to cessation of growth or shrinkage at around midday. Growth rate varied under different seasonal and environmental conditions and between stolons of the same plant. The initiation of stolon tip swelling always occurred in the late afternoon or early evening. This was the first report of the precise timing of the commencement of stolon swelling in potatoes. The timing of swelling initiation coincided with the timing of the rapid growth of stolons and tubers in the diurnal cycle.

Tissue turgor in swelling tips was always higher than in elongating stolons measured during the rapid plant growth period. Under inductive conditions, stolon turgor at midday was found to be higher than under non-inductive conditions. This provided evidence that turgor may be part of the stimulus for potato tuber initiation. Further evidence supporting this theory was gained in an experiment where stolon water potential was altered over a short period when initiation of swelling had just

commenced in a hydroponic crop. Stolon turgor at the period of rapid elongation was decreased by adding polyethylene glycol (PEG) to the nutrient solution or increased by replacing the nutrient solution with distilled water. Rate of tuber initiation was decreased in the PEG treatment and increased in the distilled water treatment compared with standard nutrient solution.

This study demonstrated that NFT hydroponics is a valuable system for the whole plant study of potato tuber initiation. The system facilitated non-destructive assessment of stolon and tuber development and also the compact size of the experimental system enabled treatments such as photoperiod and temperature to be applied. Characteristic stolon diurnal growth patterns were documented for the first time using the NFT hydroponic system, and this permitted the identification of the timing of swelling of stolon tips. Turgor potential was proposed as part of the stimulus for tuber initiation, with implications for potato crop management as manipulation of turgor may be used to influence tuber initiation. Further investigation of water relations under different inductive and non-inductive treatments will improve our understanding in this exciting new area of study in potato tuberization.

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# ABBREVIATIONS

Abbreviations and symbols are defined when first used in the text. The following list covers those most commonly used and notes variations used in papers referred to in the literature review.

| | |
|---|---|
| ABA | abscisic acid |
| AGPase | ADP glucose pyrophosphorylase |
| CCC | chlorocholine chloride |
| CHO | carbohydrates |
| GA | gibberellin |
| IAA | indoleacetic acid |
| JA | jasmonic acid |
| LD | long day |
| LM | laser micrometer |
| LVDT | linear voltage displacement transducer |
| NFT | nutrient flim technique |
| PAR | photosynthetically active radiation |
| PBZ | paclobutrazol |
| PEG | ployethylene glycol |
| RH | relative humidity |
| SD | short day |
| SE | standard error |
| SS | sucrose synthase |
| UGPase | UDP glucose pyrophosphorylase |

# CONTENTS

# CHAPTER 1

# GENERAL INTRODUCTION

## PROJECT BACKGROUND

Potato (*Solanum tuberosum* L.) is the world's fourth most important crop after the cereals maize, rice, and wheat. With high productivity per unit area, a short growing season and high market prices, potatoes generate higher returns per hectare than most other food crops (Beukema and van der Zaag, 1990). The importance of the crop is reflected in the volume of research published on all aspects of potato biology, including the discipline of physiology. A number of texts (e.g. Li, 1985; Harris, 1992; Ewing, 1997) and review papers (e.g. Wareing, 1983; Melis and Van Staden, 1984; Menzel, 1985a, b; O'Brien *et al.*, 1998) have summarized much of the research underpinning our understanding of potato plant growth and development. One key aspect of development, the initiation of tuber development on stolons, has proved difficult to investigate in intact plants because the below-ground growth and development of tubers are difficult to monitor under normal conditions of culture without destruction of the entire plant. This has constituted a major obstacle to research.

Different methods have been developed for examining the physiology of tuberization as alternatives to using field-grown plants. These include the zone-separation method, where the zone of stolon and tuber formation is separated from the soil and roots (Wurr, 1977; Krauss, 1985; Struik and Van Voorst, 1986), cuttings (e.g. Gregory, 1956; Chapman, 1958), *in vitro* culture (e.g. Palmer and Smith, 1969a, b, 1970; Perl *et al.*, 1991), solution culture (Struik and Van Voorst, 1986; Wan *et al.*, 1994) and nutrient film technique (NFT) hydroponics (Wheeler *et al.*, 1990). The zone-

separation and solution culture methods permit examination of the physiology of tuberization within whole plants, but the operation of both is time-consuming and achieving adequate replication in experiments can be problematic. Cuttings and *in vitro* techniques have therefore been the most widely used systems for examining the physiology of tuberization. These techniques allow detailed study of processes occurring at the site of tuber formation, but limit capacity to examine correlative events at the whole plant level.

The introduction of large-scale NFT hydroponic systems for commercial minituber production has provided an alternative method for studying potato physiology from whole plant level. The NFT hydroponic systems have been described by Wheeler *et al.* (1990), and can support large numbers of plants in relatively cheap and easy to operate facilities. NFT systems provide ease of access for tissue sampling for physiological and anatomical studies and are therefore ideal for examination of tuber initiation at the whole plant and organ levels.

This project investigated aspects of tuber initiation in potato using a modified NFT hydroponic system. The project was initiated by a commercial hydroponic potato minituber production business, Sunrise Seed Potatoes Pty Ltd., and was funded through an Australian Research Council Linkage Grant. Sunrise Seed Potatoes sought to increase the efficiency of minituber production through improved understanding of the physiology of tuber initiation in potatoes.

Minituber production using hydroponics, along with a number of other novel minituber production systems, has become an important component of seed potato production in developed countries in recent years. The introduction of these new technologies has decreased the number of field generations required for seed production, and reduced exposure of seed tubers to pathogens, thus improving seed quality. Minitubers are relatively easy to manage (Hussey and Stacey, 1981; Espinoza *et al.*, 1984) and can serve as an initial source of pre-basic seed stock for the certified seed potato industry (Vodenik and Jenko, 1992; Melching *et al.*, 1993).

As with all new technologies, opportunities exist to improve productivity in the NFT hydroponic minituber system. The company initiating this project was particularly interested in investigating links between nutrient uptake and tuber formation, and seasonal variations in minituber yield. An improved understanding of regulation of tuber formation in hydroponic techniques opens opportunities for manipulation of the NFT production system to increase minituber yield.

The research undertaken in this project is presented in seven chapters. Chapter 1 contains a general introduction to this project and brief review of relevant literature. Chapter 2 describes the general methods and materials used in the study. Chapter 3 focuses on the morphology of stolon development and tuber formation in hydroponics and is divided into two subchapters. Chapter 3A describes the initiation and development of stolons and tubers in NFT hydroponics in comparison with literature reports of field- and pot-grown potatoes. A series of developmental stages are defined based on these observations. Chapter 3B covers studies on the timing and rate of events during tuber formation. The relationship between rate of stolon development and tuber initiation is discussed. Chapter 4 explores the relationships between nutrient uptake and tuber initiation and describes the uptake rate of nutrient elements and water in plants at different developmental stages. Chapter 5 investigates the diurnal pattern of plant water relations and growth. This chapter includes two subchapters. Chapter 5A describes the diurnal growth pattern of stolons and tubers. The timing of swelling initiation during a diurnal cycle is documented. Chapter 5B describes the pattern of plant water status in leaves, stolons and tubers. Chapter 6 examines the effect of turgor on the growth of stolon and tuber, and on tuber initiation. The relationship between turgor and stolon development, plus turgor and tuber development, is investigated, and the role of turgor in tuber initiation is discussed. The thesis concludes with a general discussion in Chapter 7. Key findings are summarized and conclusions from the study are drawn, with further research directions suggested based on the project findings.

# LITERATURE REVIEW

## Introduction

The importance of potato as a world food crop, and of tubers as the harvestable component of the plant, has ensured that a large amount of research activity has been undertaken on tuber formation. Although a complete understanding of the processes involved in tuberization has not yet been obtained, a very large body of published data exists and many aspects of tuber initiation and development have been described. In reviewing the body of literature for this project, particular attention has been paid to factors influencing tuberization, regulation of tuberization and systems used to study tuberization. This review provided an overview of literature relevant to the study of tuberization in intact plants in hydroponics. Shorter reviews of literature specific to experimental approaches used in the study have been included at the start of each research chapter.

## Key Events in Tuberization

Under normal conditions, potato tubers form on the underground stolons, either at the main stolon tip or the tip of a stolon branch. More rarely tuberization takes place in other growth points in potato plants, such as axillaries buds (Ewing, 1995). Stolons are modified lateral shoots arising from nodes at the base of the shoot system below the soil, and consist of elongated internodes and small scale-leaves (Kumar and Wareing, 1972). While tuber formation occurs at the tips of the stolons, processes occurring in other plant organs influence tuberization. These include detection of environmental cues involved in tuber initiation and endogenous regulatory systems. The sequences of processes occurring in the plant during tuber initiation culminate in the structural and biochemical changes at the stolon tip that characterize the transition from stolon to tuber development.

**Morphological and Anatomical Changes**

The first observable sign of tuberization is swelling of the stolon in the subapical region (Xu *et al.*, 1998a). Tubers are defined as a swelling stolon tip that is more than twice the diameter of the subtending stolon (O'Brien *et al.*, 1998). The tiny swellings are sometimes referred to as tuber initials (Ewing, 1997).

The earliest anatomical change involved in the transition of a stolon to a tuber has been reported to be the reorientation of the microtubule cytoskeleton in cell walls (Fujino *et al.*, 1995; Sane *et al.*, 1996). The cell wall cytoskeleton reoriented by 90° from the longitudinal axis of the stolon permitting expansion of cells in the lateral plane resulting in swelling of the subapical region of the stolon. After reorientation, other anatomical changes occurred including cell enlargement, decreased cell division in a longitudinal direction, and an increase in cell division and cell enlargement in a lateral plane to produce tuber growth (Cutter, 1992; Koda and Okazawa, 1983; Duncan and Ewing, 1984).

The initial increase in stolon diameter at tuber initiation is most likely a result of cell enlargement, with many authors concluding that cell enlargement precedes an increase in cell division (Booth, 1963; Koda and Okazawa, 1983a; Cutter, 1992; Fujino *et al.*, 1995; Xu *et al.*, 1998a; Vreugdenhil *et al.*, 1999). In longitudinal section of swelling stolon there was little evidence of cell division (Booth, 1963; Koda and Okazawa, 1983), and the average number of cells across the pith region of the stolon behind newly initiated tubers was almost the same as in the region of the tuber itself (Booth, 1963). An *in vitro* study of early tuber initiation using excised stolon nodes indicated that for at least the first 10 days tuber enlargement was due to cell expansion in cortex and pith, not to cell division in these tissues (Peterson and Barker, 1979).

Sanz *et al.* (1996) and Vreugdenhil *et al.* (1999) found that cell division occurred in the stolon apical region at the time of tuber initiation, and concluded that this kind of

division was unlikely to be associated with swelling of the stolon as the plane of division was transverse only, leading to stolon elongation. Following the initiation of swelling, the transverse cell division in the apex stopped, and cells in the subapical region enlarged. This enlargement preceded cell division in the swelling region during the initiation of radial tuber growth.

After swelling, both cell division and expansion continued until tubers reached their final size in both *in vivo* and *in vitro* conditions (Xu *et al.*, 1998a). The relative importance of cell division and expansion to tuber growth was investigated and the rate of cell division was greater and contributed more to the increase in tuber size than that of cell expansion (Liu and Xie, 2001).

Not all tuber initials go on to develop into tubers. Some are resorbed. Tubers that are not resorbed are said to have been 'set' (Ewing, 1997). In the fullest sense tuberization is not yet completed after tuber set. After tuber set, some tubers remain small while others on the same plant continue to grow till mature (Ewing, 1997).

While a characteristic sequence of morphological changes occur in all stolons as they initiate tuber growth, tuberization is more complicated when studied at the whole plant level. At the plant or crop level, there is a remarkable asynchrony in events related to tuberization. Although the induction to tuberization changes the behavior and physiology of the whole plant, not all potential tuber sites are similarly and simultaneously directed towards the formation of a large and mature tuber (Struik *et al.*, 1999).

**Biochemical Changes**

In addition to the physical and anatomical changes there are number of biochemical changes that occur before and during tuberization. Some of the main biochemical changes include starch deposition (Plaisted, 1957), patatin accumulation (Paiva *et al.*, 1983), enzymatic shift from invertase-dominated to sucrose synthase-dominated

sucrose break down pathway (Ross *et al.*, 1994) and changes in hormone levels (Koda and Okazawa, 1983; Xu *et al.*, 1998b).

Starch

The appearance of starch has been correlated with the onset of tuber initiation (Lovell and Booth, 1967), with the timing of starch synthesis preceding cell expansion (Hannapel, 1991). Although starch has been found at low concentrations in non-swelling stolon tips, a rapid increase in starch concentration is observed immediately after tuber initiation (Hawker *et al.*, 1979). The percentage of starch in the dry matter increased rapidly during early tuber growth and then either stabilized or increased at a slower rate (Snyder *et al.*, 1977). Rapid starch accumulation coincided with the reorientation of the cortical microtubules and altered orientation of cell division at the onset of tuberization (Sanz *et al.*, 1996; Xu *et al.*, 1998a).

Patatin

Patatin is a major soluble tuber-protein (Racusen and Foote, 1980; Paiva *et al.*, 1983). Patatin in potato plants is usually restricted to tubers and stolons associated with developing tubers (Paiva *et al.*, 1983; Rosahl *et al.*, 1987; Rocha-Sosa *et al.*, 1989). Patatin has been reported to be involved in tuberization (Paiva *et al.*, 1983; Park *et al.*, 1985) as it is absent in stolon tips from non-induced plants, but rapidly increases during the first stages of tuberization. Park *et al.* (1985) considered patatin as a reliable marker for the molecular events leading to tuberization, but a role for patatin in tuber induction has not been identified.

Enzymes

Tuberization is characterized by an apparent switch from an invertase-dominated sucrolytic system to one dominated by sucrose synthase (SS)(Ross *et al.*, 1994; Appeldoorn *et al.*, 1997). Ross *et al.* (1994) reported that sucrose synthase activity increased rapidly in the swelling stolon tips during tuberization, being nearly 20 fold higher than in non-tuberised stolons. Invertase activity was high in non-tuberised

stolons, particularly in the sub-apical region, just prior to any visible swelling, but decreased 10 fold on tuberization. It is well established that invertase activity is normally high in rapidly elongating tissues (Blanc, 1983), whereas sucrose synthase activity is high in developing tubers (Pressey, 1969). The changes in carbohydrate metabolism have only been observed after tuber initiation has commenced, so it is apparent that other factors are involved in the regulation of tuberization.

Hormones

Hormone levels and activities change drastically during tuber initiation. Gibberellin (GA) levels and activities in both aboveground and underground parts are high in plants grown in non-inductive conditions (long days (LD), high temperature) but decrease in inductive conditions (short days (SD), low temperature) (Struik *et al.*, 1999). GA levels in elongating stolons are higher than in swelling tips (Okazawa, 1967; Koda and Okazawa, 1983a). In an *in vitro* study, GA levels decreased in auxillary buds prior to tuber initiation (Xu *et al.*, 1998b). Levels of the major cytokinin in potato leaves, zeatin riboside, were 29 % higher in extracts from induced than non-induced tissue (Mauk and Langille, 1978). Endogenous levels of indoleacetic acid (IAA) were low in stolons prior to tuberization but increased at the time of tuber initiation (Okazawa, 1967; Obata-Sasamoto and Suzuki, 1979a, b). Activities of IAA were elevated in plants subjected to tuber-inductive conditions (Okazawa, 1957; Palmer and Barker, 1972, 1973). The jasmonic acid (JA) related compounds 11-OH-JA and 12-OH-JA in stolons of plants grown under SD conditions were about 10 times higher than that of plants grown under LD conditions (Yoshihara *et al.*, 1996).

These and other observations have implicated plant hormones, and particularly the GAs, in the regulation of tuberization. Fluctuations in hormone concentrations have been the main biochemical change recorded prior to the initiation of tubers. While hormones have been identified as key substances in tuberization, other factors have also been shown to influence the initiation of tubers.

# Factors Influencing Tuberization

Tuberization has been shown to be influenced by a number of environmental factors, inorganic nutrient availability and plant growth substances. Short days (Garner and Allard, 1923; Ewing 1981; Wheeler and Tibbitts, 1997; Machackova *et al.*, 1998), low temperature (Gregory, 1965; Marinus and Bodlaender, 1975; Gopal *et al.*, 1998) and high irradiance (Bodlaender, 1963; Menzel, 1985c; Demagante and Vander Zaag, 1988) promote tuber initiation and development. There are few published reports on effects of nutrients other than nitrogen on tuber initiation (O'Brien *et al.*, 1998) and the prevailing view is that tuber initiation is delayed with high nitrogen supply (Krauss, 1985). Of the plant hormones, only GAs have consistently been shown to inhibit tuber initiation, while contradictory findings have been reported for all other growth substances.

## Environmental Factors

### Photoperiod

Photoperiod has a profound impact on tuber initiation and consequently tuber yield. Tuber initiation occurred earlier in SD (less than 12 hours) than in LD (more than 12 hours) (Gregory, 1956; Bodlaender, 1963; Ewing, 1990; Ewing and Struik, 1992). In SD, the partitioning of dry matter to tubers was greater and the active tuber growth period was reduced compared with LD.

The signal of photoperiod was perceived by the leaves (Gregory, 1965). Phytochrome has been implicated in the photoperiod signal, as far-red light partially reversed this process and night break lighting with far red light inhibited tuberization (Batusi and Ewing, 1982).

There are conflicting reports surrounding the effects of photoperiod on the formation of microtubers *in vitro* plantlets. Garner and Blake (1989) reported that SD promoted

earliness of tuber formation on *in vitro* as described for whole plants, conversely, it has been shown that LD either promoted (Hussey and Stacey, 1981; Wheeler *et al.*, 1988) or had no effect (Hussey and Stacey, 1984) on tuberization. Other studies found that tuberization of some cultivars occurred under 24-hour light *in vitro* (Hussey and Stacey, 1981) and *in vivo* (Wheeler and Tibbitts, 1986, 1987; Wheeler *et al.*, 1991).

Extension of photoperiod with a mixture of fluorescent and incandescent lamps, which gave a high level of irradiance, was found to be much less harmful to tuberization than that of only incandescent lamps, which gave a very low level of irradiance (Wheeler and Tibbitts, 1986; Lorenzen and Ewing, 1990). Using cool white fluorescent lamps, at photosynthetically active radiation (PAR) of 5 μmol $m^{-2}s^{-1}$, the inhibitory effect was slightly less than using incandescent lamps at the same irradiance (Wheeler and Tibbitts, 1986).

The responses of potato plants to photoperiod has also been shown to interact with temperature. Under SD, low day/high night temperatures tended to increase shoot branching, forming a vigorous shoot system, while high day/low night temperatures led to reduced shoot growth. The effect of the temperature treatments was much reduced under LD (Steward *et al.*, 1981).

Light

Generally, low light levels during the day decreased the induction to tuberize (Bodlaender, 1963; Demagante and Vander Zaag, 1988), and subsequently the number of tubers set (Taylor, 1972; Menzel, 1985c) and tuber yield (Sale, 1973, 1974; Menzel, 1982) while promoting stem elongation and haulm growth (Bodlaender, 1963). Stolon initiation was delayed slightly by shading, but stolon number was not affected by shading (Struik, 1986). The effect of low light intensity on growth resemble the effects of high temperature, and the promotive effects of high levels of irradiance can ameliorate the inhibitory effects of high temperature (Jackson, 1999).

Temperature

The effect of temperature on growth and development in potato has been extensively studied and it can be generally concluded that a temperature range of 15 to 20 °C is optimal for tuber formation (Cutter, 1992; Burton, 1981; Ewing, 1981; Lorenzen and Lafta, 1996; Geigenberger *et al.*, 1998). Tubers rarely form at temperatures above 30 °C (Menzel, 1985a). Borah and Milthorpe (1963) reported that temperature had a much greater effect on the number of tubers than on yield, hence, the main effect seemed to be on tuber initiation rather than on subsequent growth of tubers. High temperatures have, however, been shown to retard tuber growth as well as initiation (Menzel, 1982).

High temperature increased growth of aboveground parts, including internode number, leaf number and dry matter yield of leaves and stems but reduced tuber yield (Ewing, 1981; Struik *et al.*, 1989a, b, c; Wolf *et al.*, 1990; Ezekiel and Bhargava, 1997) indicating a greater partitioning of resource to the above-ground portions of the plants. Consistent with this hypothesis, the dry weight ratio of shoot to underground part decreased from 3.7 at 20/15 °C to 1.7 at 33/25 °C day/night temperature (Bensalim *et al.*, 1998), even though total plant dry weight was not affected by temperature (after 6-weeks treatment period) (Wolf *et al.*, 1990).

High soil temperature not only decreased the accumulation of dry matter in tubers, but also led to production of small, malformed tubers (Yamaguchi *et al.*, 1964; Epstein, 1966; Reynolds and Ewing, 1989). Studies have also shown that high night temperature had a greater effect in reducing tuber yield than high day temperatures (Gregory, 1954; Went, 1957; Bodlaender, 1960, 1963; Menzel, 1982), while low night temperature reduced tuberization or had no effect on tuber number in a range of cultivars and potato species (Alvey, 1965; Moreno, 1970; Roca-Pizzini, 1972).

An early study of Werner (1934) found that tuber formation started at about 24 days after emergence under a 10-hour photoperiod, but was delayed to 45 days after

emergence under an 18-hour photoperiod at 17 °C. When temperature was raised, tuberization was delayed and did not occur at all under a 15-hour photoperiod at 27 °C (Werner, 1934). However, tuberization was induced at 27 °C or higher when N application was withheld from the nutrient solution and by reducing photoperiod to 12.5 hours or less (Werner, 1935). Gregory (1954) further investigated the relationship between photoperiod and temperature and found that tubers were initiated under SD over a wide range of day temperatures (17 - 30 °C), while in LD, the day temperature range was greatly restricted (17 °C) with the necessity for lower night temperatures (10 °C). This was similar to the results of Bodlaender (1960) and Went (1957) with different cultivars. More recently, the interaction of photoperiod and temperature has been shown by Vandam *et al.* (1996), who found that high temperatures gave lower absolute tuber growth rate, but photoperiod did not affect absolute tuber growth rate at lower temperature.

$CO_2$

Mingo-Castel *et al.* (1974; 1976) reported that high concentration of $CO_2$ stimulated tuberization of potato stolons cultured *in vitro*. Treating the potato root systems with high levels of $CO_2$ resulted in an increase in dry matter content as early as two days after treatment. When the treated plants were allowed to grow for a period of three to six weeks there was a large increase in tuberization (Arteca, 1996). $CO_2$ treated plants exhibited an increase in stolon length, number of tubers per stolon and overall dry weight (Arteca *et al.*, 1979). $CO_2$ enrichment to potato plants of cultivar Denali in controlled environment rooms increased tuber yield when daylength was 12 hours, but increased shoot growth when daylength was either 12 hours or 24 hours (Wheeler *et al.*, 1988). In a subsequent study it was shown that $CO_2$ applied to the root zone of potato plants modified endogenous plant growth substance concentrations (trans-zeatin, zeatin riboside, IAA and ABA) (Arteca *et al.*, 1980), which could possibly have acted as a trigger resulting in tuberization and increased dry matter content (Arteca *et al.*, 1979).

Water availability

Moist conditions favour stolon development (Clark, 1921). The effect of water stress on potato growth and development was reviewed by Van Loon (1981). Briefly, drought at early stages of crop growth delayed tuber initiation considerably but shortened the tuber formation period; drought at the beginning of tuberization resulted in a limited number of tubers per plant and more malformed tubers; drought during tuber bulking encouraged crop senescence. Struik and Van Voorst (1986) reported that the formation of tuber initials was greatly enhanced when the stolon medium was kept air dry during tuberization. This effect was more pronounced when uptake of water by the roots was sufficient. However, the large increase in the number of swollen stolon tips, caused by the dry stolon medium, did not result in more tubers. Field experiments on the effects of drought on tuber initiation and development have produced contradictory results (Ewing and Struik, 1992). Soil moisture stress during tuber initiation in pot experiments decreased tuber number but similar stress after tuber initiation did not affect tuber number per stem (MacKerron and Jefferies, 1986). Early drought stress also reduced tuber number in long-term field studies. The relationship between rainfall and tuber number was linear during the 12 seasons examined (Haverkort *et al.*, 1990). Drought stress before stolon initiation reduced stolon and tuber numbers per stem in pot experiments but later drought stress had no effect (Haverkort *et al.*, 1990).

Nutrient elements

The effects of nitrogen on tuber initiation have been extensively studied, while other element nutrients have received relatively little attention. The vast majority of published papers concerning the effects of nutrients have assessed their effects on potato tuber yield and generally not on key stages of plant development, such as tuberization.

It can be concluded that high nitrogen applications delay tuber initiation while low nitrogen applications promote tuberization (Stallknecht and Farnworth, 1979; Liu and

Yie, 1982). Tuberization was shown to be inhibited in solution culture by continuous supply of nitrogen but occurred when the nitrogen supply was temporarily interrupted (Krauss and Marschner, 1971, 1976, 1982). Repeated cycles of high nitrogen/ nitrogen withdrawal can result in the formation of chain tubers, demonstrating that nitrogen levels play an important role in the control of tuber formation (Krauss, 1985). Under non-inductive conditions such as LD or high temperatures, reduction in nitrogen levels did not result in tuberization, indicating that nitrogen is probably not involved in the induction of tuberization but it is able to repress tuber formation when conditions are favorable for induction (Menzel, 1985b). Nitrogen supply has also been shown to influence tuber size, with reduced total availability of nitrogen resulting in small microtubers in *in vitro* tuberization studies (Garner and Blake, 1989).

The source of nitrogen has also been shown to affect microtuberization. Ammonium supplied in ratio to nitrate greater than 2:1 proved inhibitory effect to tuberization (Graner and Blake, 1989). Ammonium has also been shown to inhibit tuberization of etiolated sprout sections cultured *in vitro* (Koda and Okazawa, 1983b).

High nitrogen supply to leaves did not prevent tuberization, even though the nitrogen content of the plants was comparable to those receiving high nitrogen through the roots (Sattelmacher and Marschner, 1979). Foliar application of N had less effect on ion uptake and distribution than soil/root applications. It is likely that the form of N supplied to plants influenced uptake of other nutrients (Baker and Maynard, 1972; Kirkby and Mengel, 1967). Nitrogen source influenced the mineral composition of potato tissues. A continuous supply of $NH4^+$-N increased plant tissue P, while continuous supply of $NO_3^-$-N lowered plant tissue P (Davis *et al.*, 1986). $NH_4^+$-N also decreased the concentrations of Ca and Mg in root and shoot tissue compared to plants grown on $NH_4^+$ / $NO_3^-$ or $NO_3^-$-N (Davies *et al.*, 1986). Hence, the effect of N on potato tuberization may be partially through its effects on other ions (e.g. Ca and Mg).

It was reported that tuber yield of potato cultivar Kufri Chandramukhi increased when $K_2O$ was applied at rates of up to 50 kg/ha (Dubey *et al.*, 1997). Naik and Sarkar

(1998) showed that K had no inhibitory effect on microtuber number in the early cultivar Kufri Ashoka when applied at levels less than 25 mM. At K levels higher than 25 mM the number of microtubers in the late cultivar Kufri Sindhuri decreased. Although the amount of tubers initiated was lower at the high K levels, total microtuber mass in both cultivars increased.

In an *in vitro* study, Balamani *et al.* (1986) reported tuber numbers of 0, 14, 71 and 865 when $CaCl_2$ was applied at concentrations of 0.1, 0.3, 1 and 5 mM respectively. In addition to this the authors found that no tuberization occurred at 1 mM $MgCl_2$ alone, while $MgCl_2$ together with $CaCl_2$ promoted tuberization (Balamani *et al.*, 1986). Therefore it was concluded that $Ca^{2+}$ alone played a role in the tuberization, and that $Mg^{2+}$ may not play a promotive role in tuberization, but Mg activates several Ca-regulated enzymes such as protein kinases that promote tuberization (Balamani *et al.*, 1986).

More recently, Paiva *et al.* (1997) reported that potato cultivar Baraka plantlets grown in nutrient solution did not tuberize at all when Ca was absent, but when Ca concentrations were less than 36 mg per litre symptoms of Ca deficiency were obtained and plants produced large numbers of small tubers.

Increased Mg supply from 0 to 0.5 mM increased the dry weight of both aboveground and underground parts of potato plants (Fong and Ulrich, 1974). Plant leaf area and tuber dry weight increased with increased Mg concentrations up to 1 mM in solution, and then decreased with further increases in Mg concentrations (Cao and Tibbitts, 1992). When the Mg concentration was 1 mM in solution, the rate of $CO_2$ assimilation was higher and the dark respiration rate was lower. The same authors also found that the different Mg treatments influenced accumulation of other minerals in leaves. Leaf concentration of Ca and Mn decreased with increased Mg supply and leaf K was lower at higher Mg treatments.

The effect of nutrient solution pH on tuberization in hydroponics was studied by Wan *et al.* (1994). These authors found that at solution pHs of 3.5, 4.0 and 5.5, the average

tuber numbers per plant were 140, 40 and 2 respectively. This result suggests that the external pH might play an important role in potato induction and tuberization.

In the investigation into the role of sucrose in potato microtuber production *in vitro*, Khuri and Moorby (1995) measured the pH of base medium weekly. These authors found that the pH of the culture medium fell gradually and reached the lowest point in the fifth week, and microtuber began to form after 6 weeks. The pH was the lowest in 8 % sucrose media in the fifth week. Unfortunately these authors did not investigate the possible role of pH on microtuberization. It is possible that low pH triggers the early tuberization as in the experiment of Wan *et al.* (1994).

**Metabolites**

Carbohydrates

A number of early studies examined the relationship between endogenous carbohydrate (CHO) and potato tuberization. High endogenous CHO levels generally coincide with tuber initiation. Burt (1964) recorded an increase in soluble sugars in stolon tips and leaves as plants approached tuber initiation. Tuber initiation was associated with a three- to four-fold increase in the concentration of soluble sugars in the stolon tips and two-fold increase in leaves. Similar increases in CHO levels in stolon tips during tuberization have been reported by Hawker *et al.* (1979), Renz *et al.* (1993) and Misra *et al.* (1994). Stolon sucrose level was increased by antisensing ADP-Glc pyrophosphorylase to prevent starch formation, resulting in an increased number of tubers both per plant and per stolon (Muller-Rober *et al.*, 1992).

The effects of exogenous application of sucrose on *in vitro* microtuberization have been widely studied. Most studies have shown that a high sucrose concentration (8 – 12 %) favored tuberization when compared with low sucrose concentration (1 – 4 %) (Mes and Menge, 1954; Wang and Hu, 1982; Hussey and Stacey, 1984; Abbott and Belcher, 1986; Xu *et al.*, 1998b). Under very high sucrose concentration (10 – 20 %) potato shoots developed tubers even in darkness (Gregory, 1965). However, it is

commonly accepted that sucrose is required for but does not induce tuber formation in potato (Menzel, 1985b; Ewing, 1990).

In intact potato plants, it has been concluded that assimilates may provide part of the stimuli for tuber formation (Ewing and Struik, 1992), probably in interaction with hormones (Vregdenhil and Helder, 1992).



Growth Substances

Many growth substances including gibberellins, cytokinins, abscisic acid, ethylene and jasmonic acid have been reported to affect tuberization. The effects of these growth regulators on tuberization were reviewed by Napier and Venis (1990) and Ewing (1995).

Gibberellins

The most widely reported hormonal effect on tuberization is the inhibitory effect of the gibberellins (GAs). Decreased GA activity has been documented when leaves were exposed to tuber inductive SD (Okazawa, 1960; Racca and Tizio, 1968; Pont-Lezica, 1970; Railton and Wareing, 1973; Kumar and Wareing, 1974), and increased activity documented under non-inductive conditions, such as low irradiance (Woolley and Wareing, 1972), high temperature (Menzel, 1983) and continuous supply of nitrate in the hydroponic solution (Menzel, 1983).

During the process of tuberization GA activity has been shown to decrease in the stolon tip at the earliest stage of swelling (Smith and Rappaport, 1969; Koda and Okazawa, 1983a). After tuberization, the GA content decreased rapidly (Racca and Tizio, 1968). More recently, Xu *et al.* (1998b) found that endogenous $GA_1$ level was high during stolon elongation in 1 % sucrose medium and decreased when stolon tips started to swell under inductive conditions in 8 % sucrose medium. These findings suggest that there is a relationship between decreasing levels of GA activity and tuber initiation, and that $GA_1$ is likely to be the active GA inhibiting tuber formation.

There have also been many studies on the effects of exogenous applications of GAs on tuberization. It has been consistently shown that application of GAs to shoots, or cuttings, delayed or inhibited tuber initiation but enhanced shoot growth (e.g. Lippert *et al.*, 1958; Lovell and Booth, 1967; Moser and Hess, 1968; Kumar and Wareing, 1974; Menzel, 1980; Xu *et al.*, 1998b), while GA applied to tubers or stolons inhibited tuber development and promoted the formation of new stolons (Krauss and Marschner, 1976; Mares *et al.*, 1981).

Chlorocholine Chloride and Paclobutrazol

Chlorocholine chloride chloroethyl-trimethylammonium chloride, usually refered to as CCC, is a GA inhibitor that when applied to foliage decreased the growth of stems, leaves and stolons, and promoted tuberization (Dyson, 1965; Dyson and Humphries, 1966; Gifford and Moorby, 1967; Dutta and Kaley, 1968). CCC treated potato plants also exhibited increased tuberization when grown under non tuber inductive LD conditions and CCC treatment completely reversed the inhibitory effect of high temperature on tuber production (Kumar and Wareing, 1974; Menzel, 1980). CCC has also been found to be effective in the stimulation of tuberization *in vitro* (Harvey *et al.*, 1991; Simko, 1991, 1993; Levy *et al.*, 1993), and in increasing the number of microtubers at the early stage of culturing (Yamamoto and Nakata, 1997).

Paclobutrazol (PBZ), a triazole compound that inhibits GA synthesis, inhibits extension growth in a wide range of species (Davis *et al.*, 1988). PBZ promoted potato tuberization both by increasing tuber number per plant (Bandara and Tanino, 1995), bringing forward early tuberization and increasing the percentage of plants forming tubers (Simko, 1993), while it inhibited stem growth in greenhouse plants (Balamani and Poovaiah, 1985) and *in vitro* plantlets (Simko, 1991, 1993). PBZ has been reported to either increase (Simko, 1991) or have no effects on tuber yield (Bandara and Tanino, 1995).

## Cytokinins

There are some contradictory reports on the effect of cytokinins on tuberization. Early papers suggested that cytokinins were the stimulus for tuberization (Madec, 1963; Courduroux, 1966), as they stimulated cell division (Skoog and Miller, 1957), inhibited cell elongation (Vanderhoef and Key, 1968), and promoted cell expansion (Scott and Liverman, 1956). The levels of the major cytokinin zeatin riboside in potato leaves have been shown to be 29 % higher in extracts from induced than non-induced tissue (Mauk and Langille, 1978; Arteca *et al.*, 1980), and reached maximal levels in shoots and underground tissue 4 days and 6 days respectively after first exposure to tuber inducing conditions (Langille and Forsline, 1974; Forsline and Langille, 1975). However, when cytokinins were applied to growing stolons of non-induced plants, there was either no effect (Krauss and Marschner, 1976) or the stolon was converted into a leafy shoot (Kumar and Wareing, 1972). The conclusion drawn from *in vitro* studies has been that cytokinins induce tuberization (Palmer and Smith, 1969b, 1970; Smith and Palmer, 1970; Pelacho and Mingo-Castel, 1991).

## Abscisic Acid

Abscisic acid (ABA) is generally considered as a tuberization promoter, because the application of this hormone has been found to increase potato tuber growth (El-antably *et al.*, 1967; Menzel, 1980; Xu *et al.*, 1998b), and promote tuberization in non-induced tissue of intact plants (Menzel, 1980), leaf cuttings (Biran *et al.*, 1974), isolated stem segments (Wareing, 1978) and stolons (Krauss and Marschner, 1976), and plants grown in solution culture under non-inductive conditions of continuous high nitrogen supply (Krauss and Marschner, 1976).

It has been reported that ABA could be directly active at the tuberization site, but that its function was to inhibit the activity of the apical meristem (Wareing and Jennings, 1980). This is in agreement with the finding that ABA application inhibited stolon elongation in potatoes (Palmer and Smith, 1969a).

Not all studies have reported tuber initiation following ABA application. ABA treated plants either displayed no effect or prevented tuberization *in vivo* and *in vitro* (Palmer and Smith, 1969b; Smith and Rappaport, 1969; Claver, 1970). Furthermore, a wilt mutant of potato with highly reduced levels of ABA was found to form tubers (Quarrie, 1982). This finding made it questionable whether ABA was required for tuber initiation under natural conditions. It was also reported that there was a poor correlation between endogenous levels of ABA and tuberization (Kumar and Wareing, 1974; Wareing and Jennings, 1980). It appears that the role of ABA is primarily an indirect one and that it acts by suppressing shoot growth, and making more CHO available for storage in the tuber. This is consistent with the finding that the endogenous ABA levels in the shoots increased when tuber-forming plants are subjected to tuber inductive conditions.

Ethylene

Ethylene may play a role in tuberization in potato. Ethylene treatment to sprouts led to inhibition of extension growth, restriction of root development and swelling of stolons, stems and axillary buds (Catchpole and Hillman, 1969). Endogenous ethylene levels peaked one week before the onset of tuberization in Dahlia under SD treatment (Braine *et al.*, 1972) and promoted tuber initiation, while inhibiting the later stages of tuber filling when applied to potato plants (Halevy and Brain, 1975). However, a number of contradictory results have been reported in the literature on the effect of ethylene on tuberization. Some described the effects of ethylene on stolon growth rather than on tuberization (Palmer and Barker, 1973). Preventing gaseous exchange by enclosing plants in a culture vessel transformed leafy shoots into thick stoloniferous shoots with scale-like leaves (Hussey and Stacey, 1981). Ethylene explosure has also been shown to inhibit tuberization in *in vitro* cultures (Hussey and Stacey, 1984). The effect was reversed by adding ethylene absorbing agents to the culture vessel.

Auxins

Auxins, such as IAA and NAA, increased the number, size and sometimes the earliness of tubers when applied to whole plants and cuttings, stem segments, stolons, sprouts and tubers (reviewed by Menzel, 1985b).

Endogenous levels of indoleacetic acid (IAA) in potato tissues were estimated by Okazawa (1967) and Obata-Sasamoto and Suzuki (1979a, b) to be low in stolons prior to tuberization, but increased at the time of tuber initiation. In contrast, results of evaluations of peroxidase and IAA oxidase indicated that IAA might decrease at the time of tuber initiation. Activities of these auxin degrading enzymes were elevated in plants subjected to tuber-inductive conditions (Okazawa, 1957; Palmer and Barker, 1972, 1973).

Harmey *et al.* (1966) found that the addition of IAA to the *in vitro* culture medium produced earlier and heavier tubers *in vitro* than in untreated controls. This effect was only found in the presence of a high level of sucrose in the medium. At low sucrose levels IAA was unable to stimulate tuber initiation but gave rise to attenuated shoots. Incorporation of IAA in the culture medium has been demonstrated to induce tuberization of stem sections and axillary shoots cultured *in vitro* (Stallknecht and Farnsworth, 1982). Studies using [$^{14}$C] IAA showed that IAA was not moved upward into the shoot tissues that were embedded in the amended medium (Krauss, 1985).

The incorporation of NAA into *in vitro* media resulted in stimulation of tuber formation and tuber initiation in cultured plantlets (Stallknecht and Farnsworth, 1982). The results of NAA on tuberization of axillary shoots were similar to those exhibited by IAA, but the percentage of shoots tuberizing was extremely variable (Krauss, 1985). In contrast to IAA, NAA at the concentrations studied effectively inhibited coumarin-induced tuberization. The suggested mode of action of NAA with regard to tuber initiation was to affect the endogenous cytokinin concentration (Tizio and Biain, 1973).

Not all studies have demonstrated a promotive effect of auxins on tuberization. Exogenous application of IAA has been reported either to inhibit or to fail to induce tuber initiation in some studies (e.g. Harmey *et al.*, 1966; Kumar and Wareing, 1974). The basis of the difference in response to auxins in whole plant and *in vitro* systems has not been examined.

Jasmonates

Compounds related to jasmonic acid (JA) are thought to act as tuber promoters as they demonstrate tuber-inducing activity *in vitro* (Koda *et al.*, 1991). The active compound, an aglycone, was named 'tuberonic acid' (Ewing, 1995). Martin-Closas *et al.* (1997) reported that explants treated with JA at 30 °C showed a significant increase in tuberization, regardless of photoperiod length. The role of the JA during tuberization may be its capability of inducing expansion of cells. Takahashi *et al.* (1994) reported that JA and air-borne methyl jasmonate (JA-Me) are capable of inducing expansion of potato cells. Light microscopy revealed marked swelling of potato tissue discs after 1 day of culture in medium contained JA, with the growth due to expansion and not division of the cells (Takahashi *et al.*, 1994).

The hydroxylation of JA was affected by daylength (Helder *et al.*, 1993b). Under SD conditions both 11-OH-JA and 12-OH-JA (TA) were found in the leaves of a wild-type potato (*Solanum demissum*) that had formed tubers. Under LD conditions tuberization did not occur and these compounds were undetectable. It was suggested that daylength controls hydroxylation of JA, thereby controlling tuberization. Yoshihara *et al.* (1996) suggested that photoperiod affected the transport of TA glucoside. These authors found that radioactivity labeled TA glucoside metabolized from [$^{14}$C] JA in stolons of plants grown under SD conditions was about 10 times higher than that of plants grown under LD conditions.

In contrast, Jackson and Willmitzer (1994) declared that JA did not induce tuber formation under LD conditions, and concluded that JA itself was not the transported

tuber-inducing signal. Helder *et al.* (1993b) also reported that application of salicylhdroxamic acid, an inhibitor of one step in the JA biosynthetic pathway, did not prevent tuberization in SD conditions. These results indicate that differences in the levels of JA itself may not influence tuberization.

## Regulation of Tuberization

Early investigators attributed the process of tuber formation to the existence of surplus carbohydrates. Wellinsiek (1929) introduced a form of the nutritional theory for tuberization. The same author suggested that the causal factor of tuberization was the concentration of metabolites from photosynthesis, especially the carbohydrate-to-nitrogen ratio. The hypotheses to explain tuber induction was that SD inhibited shoot growth, causing assimilate to be accumulated in the stolon tips, which in turn led to tuberization (Wellensiek, 1929; Werner, 1934; Burt, 1964). However, short term observations showed that this is not the case, because moving plants from LD to SD did not cause a decrease in rate of leaf expansion until well after a strong tuber sink had been formed (Lorenzen and Ewing, 1990). A role for sucrose in tuber induction cannot be discounted, however, as during tuber initiation, more assimilate was produced by the leaves and exported to stolons, and the stolons contained more sucrose under SD conditions than LD conditions (Ewing, 1990).

The dry matter distribution within potato plants is altered by photoperiod. The inductive factor SD stimulated early tuber formation but reduced foliage growth (Burton, 1989). Hence there is a relationship between tuber formation and dry matter distribution. Dry weight of leaves under SD was between a third and a ninth of those under LD conditions (Caesar *et al.*, 1981). The effect of photoperiod on dry matter distribution could also be seen from the diurnal changes in leaf starch concentration of newly mature Norland potato leaves. Leaf starch concentration decreased more rapidly

at night in SD than LD conditions, showing more effective carbon output under SD compared to that of LD or continuous lighting (Stutte *et al.*, 1996). Lorenzen and Ewing (1992) also showed that starch accumulation in potato leaves was greater under LD than SD, even with the presence of strong sink tubers.

The high temperature inhibition of tuberization is thought to be mediated through reduced dry matter partitioning to underground parts. Elevated temperatures decreased the activity of SS and ADPglucose pyrophosphorylase (AGPase) in tubers (Kraus and Marschner, 1984; Lafta and Lorenzen, 1995). Given that SS is closely associated with sink strength of potato tubers (Zrenner *et al.*, 1995), reductions in SS activity result in reduced partitioning of photosynthate to tubers and increased shoot growth (Borah and Milthorpe, 1963; Ewing, 1981; Struik *et al.*, 1989a, c), which in turn delays or inhibits tuberization.

Several studies have shown that reduced carbon import into potato tubers at high temperature was attributable to reduced sucrose mobilization in the tuber itself, and not just to a shortage of photosynthate supply (Krause and Marschner, 1984; Mohabir and John, 1988; Wolf *et al.*, 1991). The inhibitory effect of high soil temperatures to tuber growth was accompanied by increased sucrose levels in both tubers and leaves. This indicates a block of sucrose breakdown and starch synthesis in the tubers (Wolf *et al.*, 1991). Furthermore, potato starch synthesis is optimal at approximately 21.5 °C. Increasing the temperature to 30 °C led to a 50 % reduction of starch synthesis, whereas respiration rates increased 2-fold (Mohabir and John, 1988). This finding was confirmed in long-term experiments when individual tubers were subjected to 30 °C for 6 days; incorporation of labeled [$^{14}$C] assimilates into starch, as well as the starch content and the growth rate of the tubers, were significantly reduced, whereas the labeled assimilates into the sugar fraction was not affected by high tuber temperature (Krauss and Marschner, 1984).

It is not clear how nitrogen affects tuberization. One hypothesis is related to the ratio of carbohydrate to nitrogen. High levels of carbohydrate favor the formation of tubers,

and high levels of nitrogen promote shoot and root growth that would utilize much of the available carbohydrate and then reduce the amount of the available carbohydrate for tuber formation (Jackson, 1999).

The possible roles for sucrose to play in *in vitro* microtuberization include energy sources, an osmotic role and sucrose regulating hormonal levels and enzymatic activities. The role of sucrose as an osmotic agent in plants is well established. Along with its component hexoses, it can account for up to 35 % of osmotic adjustment in tobacco callus cells subjected to high sodium chloride (NaCl) stress (Gibbs *et al.*, 1989). These sugars were also found to increase dramatically in carrot (Fallon and Phillips, 1989) and tomato (Handa *et al.*, 1983) cells placed under osmotic stress. It is thought that the sucrose dissociated to increase osmotic potential within the cells.

The *in vitro* developing microtubers are sinks for sucrose in the culture medium. Hence, the effect of sucrose as an energy source for the developing tuber has been studied. Lo *et al.* (1972) cultured etiolated stolon nodes on White's medium with 2, 8 or 12 % of sucrose. The osmotic potential of each the media was maintained at a standard level using mannitol. Rapid tuberization was observed on cultures growing on 8 and 12 % sucrose. On 2 % sucrose media, tuberization was greatly delayed, and only a few small tubers were observed. Lo *et al.* (1972) concluded from this result that microtuberization was a response to the high sucrose concentration, and was independent of the osmotic potential of the medium. Garner (1987) showed that media containing mannitol as the carbon source resulted in four weeks delay of microtuberization, and by the end of the experiment (14 weeks) only a few microtubers had developed on that medium, compared with about twice as many on 4 % sucrose alone, and about 4 times as many on 8 % sucrose. This finding was also supported by Chandra *et al.* (1988), who used sucrose, glucose, fructose, mannose and mannitol, concentration ranging from 4 to 12 %, and found that 8 % sucrose was optimal for the development and growth of microtubers, while smaller microtubers were obtained on media containing glucose or fructose, and none on that with mannose or mannitol.

Appeldoorn *et al.* (1997) reported the effect of sucrose on UGPase. The authors used culture medium treatments of 8 % sucrose, 8 % sucrose plus GA, and 1 % sucrose, with induced axillary buds cultured in the treatment media. In the tuber inductive (8 % sucrose) and non-tuber inductive (8 % sucrose plus GA) treatments, the activity of UGPase was considerably higher than in the non inductive 1 % sucrose treatment. This was consistent with the enhancement of UGPase mRNA synthesis in potato leaves when incubated in the presence of high sucrose level (Spychalla *et al.*, 1994). The increase in UGPase activity after the onset of tuberization of field-grown potato plants (Sowokinos, 1976; Zrenner *et al.*, 1993) might also result from the sugar-induced effect, because sucrose content was always high in the swelling tips of stolons (Ross *et al.*, 1994).

More attention has been given to the effects of plant growth substances on tuberization since the discovery of growth substances. Driver and Hawkes (1943) suggested that the existence of a specific factor produced under favorable photoperiods in the leaves was moved to the stolons, inducing tuberization. Grafting experiments, by Gregory (1954, 1956), Chapman (1958) and Kumar and Wareing (1972) provided supporting data for the hormonal theory of tuberization. The same authors demonstrated that the occurrence of a tuberization stimulus, formed in the leaves under inductive conditions, was transmissible through graft unions and concluded that it was not a major metabolite such as a carbohydrate. Others have supported this theory (Chapman, 1958; Madec, 1963; Kumar and Wareing, 1973, 1974; Ewing, 1987).

Although about 60 years have passed since the first hormonal tuberization theory was hypothesized, the existence of a specific tuberization hormone has not yet been identified. The most consistent hormonal effects on tuberization reported are from GAs. The application of GAs to the shoots of a tuber crop enhanced shoot growth and delayed tuber growth (Lippert *et al.*, 1958; Lovell and Booth, 1967; Moser and Hess, 1968; Menzel, 1980; Xu *et al.*, 1998b). GA applied as a foliar spray to cuttings also inhibited tuberization (Tizio, 1971; Kumar and Wareing, 1974). GA application to the

tubers (Krauss and Marschner, 1976), or to the stolons (Mares *et al.*, 1981) caused the cessation of tuber growth and the formation of new stolons. Conversely, the application of GA inhibitors promoted tuberization (Bodlaender, 1964; Menzel, 1980) even under non-inductive conditions (Kumar and Wareing, 1974).

Several environmental factors involved in tuberization have been reported to be mediated through plant GA levels. It was found that tuber inhibitory higher temperatures stimulated higher levels of GA activity in potato shoots (Menzel, 1983). Treating plants or cuttings with CCC, a GA synthesis inhibitor, overcame the inhibition of tuberization caused by high temperatures (Jackson, 1999). The inhibitory effect of low light intensities on tuberization might be mediated through the same control process as high temperature, as increased GA levels in potato leaves have been recorded under these conditions (Woolley and Wareing, 1972). Nitrogen withdrawal promotes tuberization. It was found that nitrogen withdrawal affected phytohormone levels, with a reduction in GA levels and an increase in ABA levels (Krauss, 1985). In a study of sucrose on tuber initiation *in vitro*, Xu *et al.* (1998b) not only reported that tuberization was highly dependent on sucrose concentration, but also found that much higher levels of $GA_1$ in the tips of stolons growing in media with 1 % sucrose, as opposed to 8 % sucrose. These authors suggested that sucrose could modulate endogenous GA levels in the stolon tip.

## Research Techniques Used in Tuberization Studies

The physiology of potato tuberization has been previously studied using a range of techniques including: intact glasshouses/field grown plants, stem cutting carrying one or more nodes, isolated sprouts, and stolons and stem segments in tissue culture.

The cutting method has been widely used in tuberization studies (e.g. Gregory, 1956; Chapman, 1958; Vreugdenhil *et al.*, 1999) and involves taking stem cutting containing one or more axillary buds from whole plants, burying them in soil or potting mix and

leaving one or more leaves exposed to the light. The development of each buried bud into a tuber or an orthotropic shoot depends on the conditions the mother plant grew in. If the mother plant was grown under strongly inductive conditions, the buried buds develop into tubers shortly after planting (Ewing and Struik, 1992). If the mother plant was grown in non-tuber-inductive conditions, the buried buds remained dormant or developed as shoots (Ewing and Struik, 1992). The cutting propagation technique provides a large quantity of material for study and can be used to test how environmental conditions, genetic differences, the application of growth substances, and other variables affect the degree to which leaves have been induced. However, this method studies tuber development only at a single growth point, neither taking into account competition between competing sinks nor the effect of removing the section from the whole plant dynamic.

The *in vitro* plants are ordinarily grown in a sucrose medium (e.g. Palmer and Smith, 1970; Perl *et al.*, 1991; Melchiorre *et al.*, 1997). These plantlets originate from apical meristems and nodal subcultures. There is no underground portion as the entire plant is maintained in continuous darkness, favoring the formation of stolons and tubers (Ewing, 1997). Tuberization responses measured on *in vitro* plants do not seem to be reliable predictors of whole plant responses as the growth conditions are significantly different compared with the natural grown plants (Ewing, 1997).

Potato tuberization is a complex process involving the interaction of different parts of the whole plant in response to external stimuli. The study of tuberization physiology has been mainly conducted using excised tissue segments. This avoids the difficulties associated with using intact greenhouse- and field-grown plants; given that tubers form underground, it is necessary to resort to sequential harvests of different plants, rather than a series of observations on the status of tuberization in a single plant when undertaking research at the whole plant level. Many plants must be examined at each harvest to overcome the phenotypic variation (Ewing and Struik, 1992). Alternatively, a method separating the zone of stolon and tuber formation from the soil and roots was devised for intact study (Lugt *et al.*, 1964; Wurr, 1977; Krauss, 1985; Struik and Van Voorst, 1986), but this method is time-consuming.

Hydroponic systems are another technique that can be used to study whole intact plants (Wan *et al.*, 1994; Cao and Tibbitts, 1994, 1998). The advantages of such systems are that they permit free access to stolons and tubers to study growth patterns without destroying plant tissue, and also permit easy access to tissue for destructive sampling in an investigation of important physiological changes occurring during tuberization in intact plants. Additionally the system size is relatively small, making it possible to facilitate manipulation of the external environment (eg. daylength) and the chemical environment surrounding the stolons and tubers through changes in the hydroponic solution.

## Conclusion

While two main theories, nutritional and hormonal, have been widely discussed in the literature, gaps still exist in our understanding of the regulation of tuberization. A common conclusion in published work is that tuber formation is a complex process involving many internal physiological changes and the interaction of different parts of the whole plant in response to external stimuli. While studies using isolated plants are valuable in elucidating aspects of the physiology of tuberization, whole plant studies are required to understand the correlative events occurring within the plant, and to validate of conclusions drawn for studies using isolated plant parts. While in a number of cases hydroponics has been used to investigate tuber formation, emphasis has generally been on optimizing production conditions to increase tuber yield. Pioneering work in hydroponics by Krauss (1985) documented changes in hormone concentrations during tuberization under a range of inductive and non-inductive conditions, with specific emphasis on nutrition during tuberization. After these studies, the use of hydroponics declined with the development of cutting and *in vitro* systems to study tuberization. This project has reused the hydroponics to investigate key aspects of growth at the whole plant level during the initiation of tuber development.

# CHAPTER 2

# GENERAL MATERIALS AND METHODS

## OUTLINE

***The experimental work in the project was undertaken over a three year period from 2000 to 2002 and consisted of 14 major experiments. The materials and methods common to the majority of these experiments are described in this chapter. Detailed descriptions of methodology and materials specific to individual experiments are presented in the following chapters of the thesis along with the data generated in the project.***

## INTRODUCTION

A nutrient film technique (NFT) hydroponic system was used to grow potato plants in all the experimental trials. A total of 14 potato crops were grown between March 2000 and December 2002 with each crop treated as an experimental unit. Each experiment involved growth of a potato crop in the NFT system, commencing with transfer of tissue cultured potato plantlets into the hydroponic system and concluding after tuber formation had occurred. Initial research focussed on characterizing the pattern of growth of potato plants in the hydroponic system and examining the rate of nutrient uptake during tuber initiation and growth. Later experiments examined the diurnal growth pattern of stolons and tubers, and water relations during tuber formation. A list of the experiments is shown in Table 1.

**Table 1.** Overview of experiments

| Trial No. | Start | Finish | Season | Brief Trial Description |
|---|---|---|---|---|
| **1** | Mar 2000 | Apr 2000 | Autumn | Familiarization with system; Development of key parameters for measurement |
| **2** | May 2000 | Jun 2000 | Autumn- Winter | Morphological description including stolon length, number of swelling tips and tubers for establishing plant developmental stages and for studying timing and rate of stolon and tuber development |
| **3** | Jul 2000 | Aug 2000 | Winter | |
| **4** | Sep 2000 | Oct 2000 | Spring | |
| **5** | Dec 2000 | Jan 2001 | Summer | |
| **6** | Mar 2001 | Apr 2001 | Autumn | |
| **7** | Jul 2001 | Aug 2001 | Winter | Method validation for webcam system and plant water status measurement; Measuring nutrient uptake rate |
| **8** | Nov 2001 | Dec 2001 | Spring-Summer | Webcam measurement of stolon and tuber growth; Effect of PBZ on tuberization, nutrient uptake rate and plant water status |
| **9** | Jan 2002 | Feb 2002 | Summer | Effect of daylength on plant water status and stolon growth |
| **10** | Mar 2002 | Apr 2002 | Autumn | Webcam measurement of stolon and tuber growth; Measuring nutrient uptake rate and diurnal plant water status |
| **11** | May 2002 | Jun 2002 | Autumn-Winter | Measuring nutrient uptake rate and diurnal water status; Webcam measurement of stolon and tuber growth |
| **12** | Jul 2002 | Aug 2002 | Winter | Diurnal pattern of growth and water status; Tuber growth rate and turgor; Turgor in stolons, swelling tips and tubers |
| **13** | Sep 2002 | Oct 2002 | Spring | Measuring nutrient uptake rate; Diurnal pattern of water status |
| **14** | Nov 2002 | Dec 2002 | Spring-Summer | Effect of PEG on stolon growth, tuber initiation and plant water status |

# MATERIALS AND METHODS

## Plant Materials

Potato plants of cultivar Russet Burbank (*Solanum tuberosum* L.) were used in this project in all experiments. Virus free tissue cultured plantlets were initially supplied by Sunrise Seed Potatoes (Devonport, Tasmania), then subcultured for use in all experiments. All tissue cultured potato plantlets transplanted to the hydroponic system were approximately 10 cm high.

A stock of tissue culture plantlets was maintained by subculturing. Vigorously growing shoots from tissue-cultured plantlets were cut into segments containing two nodes for subculturing. The bottom leaf was excised from the stem. Segments were transplanted under aseptic conditions to new tissue culture medium. All cultures were maintained in a controlled environment room with 16 hour daylength, light intensity approximately 100 $\mu mol.m^{-2}.s^{-1}$ at the culture level, day temperature of 22 ± 2 °C and night temperature of 15 ± 2 °C. Plantlet vigour declined after 4 – 5 cycles of subculturing, so fresh plantlets were obtained from Sunrise Seed Potatoes when required.

## Tissue Culture

The tissue culture medium consisted of the following components:

| | |
|---|---|
| Murashige and Skoog salts | 4.4 g/l |
| Sucrose | 30 g/l |
| Casein enzymatic hydrolysate | 0.5 g/l |
| Ascorbic acid | 40 mg/l |
| Distilled Water | 1 litre |
| Phytagel | 2.0 g/l |

5 % KOH solution was added to adjust the medium pH to 5.8.

Tissue culture media was prepared by adding Murashige and Skoog media powder (Sigma, Sydney) and 40 mg/l ascorbic acid to 80 % of the final required volume of distilled water, stirring until completely dissolved. In order to remove ethylene, 0.5 g/l casein hydrolysate (Sigma, Sydney) was added. 3 % (w/v) sucrose was then added and dissolved. The solution was made up to the final volume, and the pH was adjusted to 5.8 using 5 % KOH. 0.2 % (w/v) Phytagel (Sigma, Sydney) was added to the solution. Phytagel has been shown previously to be a superior gelling agent than agar in media used for potato micropropagation (Veramendi et al., 1997). The solution was then heated using a microwave oven and removed just prior to reaching boiling point. A volume of 25 ml of hot liquid medium was poured into 120 ml polycarbonate tissue culture vessels (Crown Scientific Pty. Ltd., Hobart, Tasmania) and autoclaved for 15 minutes at 121 $^{o}$C and 103 kPa. Culture vessels containing medium were then cooled to room temperature, sealed and stored at room temperature for later use.

## Hydroponic Systems

NFT hydroponic systems with recirculated nutrient solution were used in all trials. Six separate systems were constructed for the project. Each system consisted of a 180 cm long white polymer coated steel tray held at a 2 % slope. The trays were 30 cm wide and 5 cm deep. The top of each tray contained a 5 cm wide white coated steel lip extending inwards from one side (Fig. 1). Holes of 20 mm diameter were drilled at intervals of 20 cm along this steel lip and the tissue-cultured plantlets were planted through these holes. A single layer of capillary matting (Sage Horticultural, Victoria, Australia) was placed on the base of each tray and an opaque polyethylene film covered the top to exclude light and maintain a high humidity in the trays. The polyethylene film was glued to the tray lip and was held to the other side of the tray using pegs. This set up enabled access to the tray by releasing the pegs and lifting the film. Nutrient solution was held in 100-litre food grade opaque plastic drums placed below the lower end of each tray. Solution was plumped to the higher end of each tray using Rio-2100 submersible pumps (TAAM, Inc., USA). All piping was 13 mm diameter black polyethylene pipe except that the outlet of the solution into the tray was 5 mm diameter

pipe. The solution reservoir was covered to prevent algal growth. Pumping rate was adjusted to supply an outlet flow rate of 125 ± 5 ml per minute.

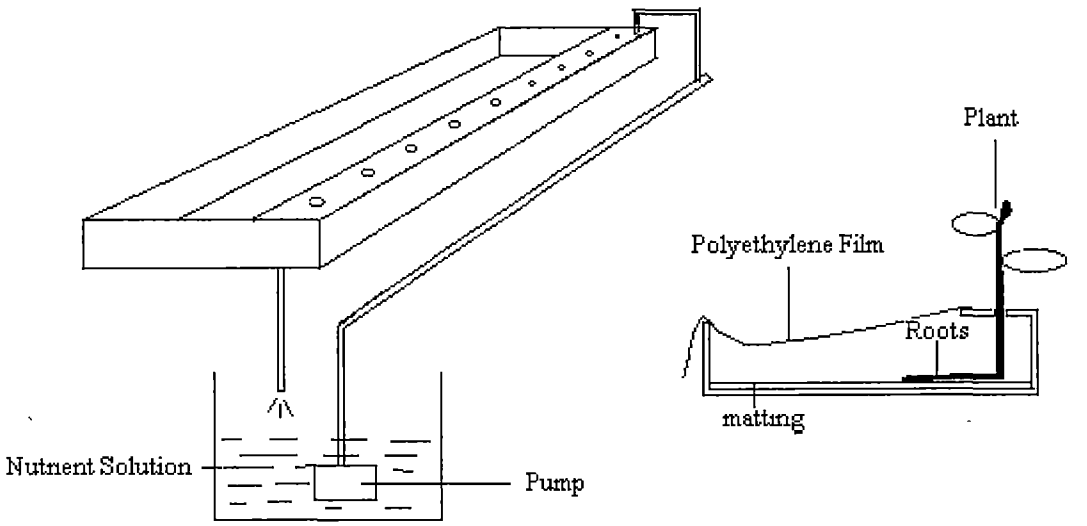


**Figure 1.** Schematic diagram of NFT hydroponic system

## Nutrient Solution

A commercially available hydroponic nutrient salt mixture (Culture-S, ACCENT Hydroponics Pty. Ltd., Australia) was used for preparing nutrient solution. Stock solutions were prepared according to the manufacturer's recommendations. The nutrient consisted of two parts, part A and part B salts. Part A was $Ca(NO_3)_2$ and part B was a combination of salts. Part A and part B salts were dissolved into fresh tap water to make 11 litre of stock solutions A and B. Stock solutions were stored in sealed light proof food grade plastic drums.

The standard nutrient solution was made using A and B stock solution. A 50:50 mix of A and B stock solutions were diluted 100 times to make the hydroponic nutrient solution. The nutrient solution was held in the greenhouse for 24 hours, adjusted to pH 5.8 using 5 % KOH or 5 % $H_2SO_4$ solution and then added to the hydroponic system.

The concentration of nutrient elements in the final solution was:

| Element | (ppm) | (mM) |
|---|---|---|
| Nitrate nitrogen | 210 | 3.39 |
| Phosphorous | 60 | 1.94 |
| Potassium | 330 | 8.46 |
| Calcium | 170 | 4.25 |
| Magnesium | 50 | 2.06 |
| Sulphur | 65 | 2.03 |
| Iron | 6.0 | 0.11 |
| Manganese | 2.0 | 0.04 |
| Boron | 0.3 | 0.03 |
| Copper | 0.06 | 0.0009 |
| Zinc | 0.06 | 0.0009 |
| Molybdenum | 0.007 | 0.00007 |

During experiments, the pH and conductivity of the nutrient solution were maintained at pH at 5.8 and electrical conductivity at 2.0 mS/cm by an automated NutriDose monitoring system (Autogrow Systems Ltd., New Zealand). The pH was adjusted using 5 % KOH or 5 % $H_2SO_4$. An equal volume of stock solutions A and B were used to adjust electrical conductivity. Nutrient solution was replaced with fresh solution on a regular basis, ranging from every 10 days when plants were first planted in trays to every 7 days when plants reached maximum size.

## Greenhouse Conditions

All experiments were carried out at the Horticultural Research Centre, School of Agricultural Science, University of Tasmania (42.9° South, 147.3° East). A single daylength experiment that was carried out in a greenhouse specifically designed for photoperiod treatments, while all other experiments used another glasshouse at the same location.

Glasshouse temperature was thermostatically maintained between 15 °C and 25 °C. The average temperature measured was 13 °C and 18.4 °C in winter and in summer respectively. The average relative humidity was 76 % in winter and 67 % in summer.

Plants were grown under natural daylength and light intensity conditions. Daylength varied from approximately 9 hours in winter to 15 hours in summer. The daily highest photosynthetic photon flux density at the plant level varied from approximately 400 $\mu mol.m^{-2}.s^{-1}$ in winter to 1200 $\mu mol.m^{-2}.s^{-1}$ in summer.

## Standard Measurements

### Plant Growth

Access to underground part organs was achieved by releasing pegs and folding back the opaque polyethylene film covering the trays. The number, length and diameter of stolons and tubers on individual plants were measured in experiments. Initially stolon length was measured using a compass and a ruler, and stolon and tuber diameter were measured using vernier callipers. A webcam system was later constructed to take time lapsed pictures from which the length of stolons and the diameters of stolons and tubers were recorded.

Plant fresh weight in the hydroponic trays was determined indirectly. Prior to planting, the hydroponic system was run for 24 hours, drained until solution ceased flowing at the tray outlet, and the weight of the hydroponic tray plus matting was measured . The weight of the tray plus plants was measured using the same method at different stages of crop growth. Plant fresh weight on a whole tray basis was obtained by subtracting the weight of the bare system from the total weight of the system with plants.

### Nutrient Uptake

The rate of nutrient uptake was recorded on a whole tray basis over a 24 hour period. Nutrient solution samples were collected from the outlet drain of the hydroponic tray at

10:00 am on one day and again at 10:00 am the following day. After accounting for water loss, the difference in concentration of nutrients between the two samples was used as a measure of nutrient uptake.

Fresh nutrient solution was added to the hydroponic trays at the commencement of each nutrient uptake measurement. The volume of nutrient solution used at the commencement of each measurement was determined by recording the weight of the solution as it was added to the reservoir. After flushing the new solution through the system for 1 hour the first solution samples were collected and weighed. The second samples were collected after 24 hours. The pump was turned off at this time and the trays allowed to drain fully before volume of nutrient solution was again determined by recording the weight of the solution in the reservoir. Nutrient solution samples were stored in polycarbonate bottles at 4 °C before analysis. Bottles were washed with 15 % HCl solution and rinsed with distilled water prior to sample collection.

Ca, Mg, K, P and S were analysed using an 'IRIS' Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Thermo Jarrell Ash, Franklin, Ma, USA). Each calibration protocol typically consisted of a blank and 3 - 5 standards covering the concentration range 0 to 200 ppm. Standard mixtures were prepared in 5 % (v/v) $HNO_3$. Standard mixture preparations (AccuStandard Inc, ICPMO143-5 and ICPMO165-5, New Haven, CT, USA) were analysed regularly to ensure accuracy. Sample uptake time to the ICP-OES was approximately 60 seconds, while the rinse time between samples was also approximately 60 seconds.

$NO_3^-$ concentrations were analysed using the ultraviolet spectrophotometric screening method at 220 μm (Cawse, 1967). The standard curve was obtained from running a series of standards containing 0 – 7 ppm $NO_3^-$, with 1 ml 1 N HCl added to each 50 ml standard solution prior to sample measurement. Acidification prevented interference from hydroxide or carbonate. One ml of each nutrient solution sample was diluted to 50 ml with distilled water and 1 ml of 1 N HCl. The value of absorbance from the

spectrophotometer was recorded and the concentration of $NO_3^-$ was calculated from the standard curve.

The concentration of $H^+$ in nutrient solution was obtained by measuring sample solution pH value using a pH meter (Denver Instrument Company, USA.). Nutrient solution samples were transferred from 4 °C storage to room temperature for approximately 2 hours before measuring.

## Water Status

### Total Water Potential

Leaf, tuber and stolon water potential were measured using the pressure chamber technique as outlined by Gandar and Tanner (1976). Measurements were taken immediately after excising the plant organs with a razor blade.

After recording water potential, the samples were placed into labelled plastic vials and snap frozen using liquid nitrogen. The samples were then transferred to the laboratory and stored at –80 °C until required for measurement of osmotic potential.

### Osmotic Potential Measurement and Turgor Calculation

Samples were thawed in room temperature for about 1 hour and centrifuged at 10000 (rpm) for 5 mins. 10 µl of extracted solution was used for measuring osmotic potential using a Wescor Model 5100C vapour pressure osmometer (Wescor Inc., Logan, UT, USA).

Turgor potential (P) was derived from the difference between water potential ($\psi$) and osmotic potential ($\pi$).

$$\mathbf{P = \psi - \pi}$$

## Diurnal Measurement of Stolon and Tuber Growth

An image capture and analysis system was developed to enable frequent, non-destructive assessment of stolon and tuber length and diameter. The system was based on image capture using a computer linked to a series of webcams.

High quality webcams (Log 0414, Logitech, PRC) able to produce 480 x 720 pixels image, were connected to a computer (PC Pentium 4, 1.6 MHz) via the USB port. Six webcames could be operated from the single computer. Software (Supervision 3.0) was installed on the computer to run the multi-webcam system. Light source plus webcam operation were synchronized to capture images of growing stolons and tubers at set time intervals.

A schematic illustration of the webcam system for capturing time-lapse images is shown in Fig. 2. Images were analysed using the freely available software ScreenRuler and Scion Image. ScreenRuler was used for measuring length while Scion Image was used to measure both area and length.

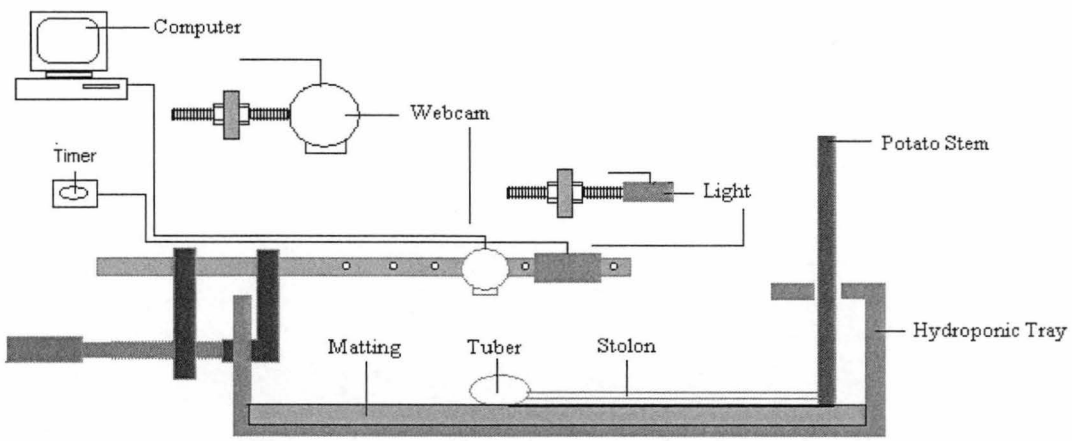


**Figure 2**. Schematic diagram of webcam image capture system and hydroponic system for measuring changes in potato stolon and tuber dimension.

The light for taking images of potato tubers and stolons was given out by a low voltage (10 watt) halogen lamp with an UV filter (Martec, PRC). This light source provided enough illumination for clear images without significantly increasing temperature. Temperature measured 10 cm under the light in the trays increased 0.3 °C during a period of 3 minutes. The period of time a light source was on never exceeded 3 continuous minutes.

When measuring stolons/tubers, webcams were set to focus from a height of about 10 cm. A scale was set next to selected stolons/tubers and lights were switched on for 1 minute at hourly intervals. Webcams were programmed to collect images when a change in light intensity was detected. Images were stored on the computer until dimension measurements using image analysis software were recorded.

## Statistics and Computing

ANOVA analysis of variance was undertaken using SAS (SAS Institute Inc. 1989). The significance between treatment means was assessed at the 5 % level using Fisher's LSD (least significant difference) where significant ($P < 0.05$) (Steel and Torrie, 1980). Pearson correlation coefficient (PROC CORR in SAS) was used in multivariate analysis. Linear regressions were undertaken using PROC GLM in SAS.

Graphs were produced using Microsoft Excel, and Microsoft word (Microsoft Office 2000) was the word processing package used in the compilation of this thesis.

# CHAPTER 3

# TUBER FORMATION IN NFT HYDROPONICS

## INTRODUCTION

Detailed morphological descriptions of hydroponically grown potato plants have not been published despite a number of authors using hydroponics to investigate tuber formation (e.g. Tibbitts and Wheeler, 1987; Wan *et al.*, 1994; Tibbitts and Cao, 1994). While it can be assumed that stolons and tubers in hydroponics will not differ significantly from published descriptions of stolon and tuber morphology in field grown or pot grown plants, a comprehensive study of tuber formation in hydroponics requires this assumption to be validated. The morphological changes occurring during the transition from stolon to tuber development can provide clues to the physiological processes regulating the formation of tubers. In particular, the timing of morphological and physiological changes during tuber formation were used in this study to investigate aspects of tuberization.

The initial focus of this project was identification of the rates and timing of stolon and tuber development, and nutrient uptake rates associated with key stages of plant development. This research required the identification of the key developmental stages involved in tuberization and the timing and rate of stolon and tuber development in hydroponics.

A review of the literature on potato stolon and tuber morphology and anatomy is made in this chapter as background to two research sections. In Chapter 3A, morphological changes during stolon elongation and early tuber formation in hydroponics are described, and a crop level scale is established. This scale was applied to hydroponically grown plants to separate crop development into discrete

stages. In Chapter 3B, the timing and rate of stolon and tuber development and the correlations between stolon, shoot and tuber development in hydroponically grown plants are presented.

# LITERATURE REVIEW

## Morphology of Stolon and Tuber Development

Detailed descriptions of stolon and tuber anatomy and morphology have been published by a number of authors (e.g. Kumar and Wareing, 1972; Peterson *et al.*, 1985; Cutter, 1992; Melchiorre *et al.*, 1997). Potato stolons are modified lateral shoots arising from nodes at the base of the shoot system, below the soil. Stolons have elongated internodes and small scale-leaves (Kumar and Wareing, 1972). Stolons differ from normal green shoots in having more elongated internodes, hooked tips, small scale-leaves, lack of chlorophyll and a diageotropic habit (Kumar and Wareing, 1972). The apical meristem displays a 'tunica-corpus' organization (Melchiorre *et al.*, 1997). The 'tunica' is composed of two layers of external cells, with the division plane being perpendicular to the meristem surface. The central region of the 'corpus' contains a mass of highly vacuolated cells surrounded by a peripheral meristem. Below this region the pith meristem accompanied by large parenchymatic cells, can be observed (Melchiorre *et al.*, 1997). During stolon growth, cell division occurs in the apex. The newly divided cells expand longitudinally, leading to stolon elongation. The development of branches from nodes on the stolon can occur as a stolon elongates. Tubers form near the growing points of stolons. During tuber initiation, stolon elongation stops and the subapical stolon region expand radially, resulting in swelling behind the stolon apical meristem. Continued radial expansion results in the development of a tuber.

Botanically, tubers are greatly shortened and thickened stems that bear scale leaves (cataphylls), each with a bud in its axil (Ewing and Struik, 1992). Basically, a potato tuber is a modified stem with degenerated leaves and axillary buds, shortened internodes, and a radially expanded stem axis (Cutter, 1992). The end of the tuber

derived from stolon apex is usually referred to as the 'rose' end or 'bud' end, and the end attached to the stolon is the 'heel' end or 'stem' end. The degenerated leaves are complete scale leaves with little height (Cutter, 1992). All leaves derived from the stolon apex are arranged in a spiral phyllotactic pattern. Their associated axillary buds are referred to as 'eyes' (Artschwager, 1924). Each 'eye' is a stem node (Adams, 1975) and consists of a main axillary bud, its associated subtending scale leaf, and axillary buds associated with the two oldest leaves formed by the main axillary bud (Cutter, 1992). Other visible small white dots on the tuber skin are lenticels (Artschwager, 1924). The colour and shape of tubers vary broadly (Mes and Menge, 1954; Burton, 1989).

Anatomically, tubers consist of four primary zones of tissue: periderm or skin, cortex, perimedullary and pith. Periderm is the outermost covering of the tuber, consisting of the phellem, phellogen and phelloderm. The cortex is located between the periderm and the vascular tissue. The perimedullary tissue lies between the vascular tissue and pith, and comprises the largest part of the storage tissue. Pith is the centre of the tuber tissue and may be angular with rays extending to each node. The vascular system contains an abundance of internal and external phloem strands but a small proportion of xylem (Cutter, 1992). The cells of the tuber are tightly packed with starch grains and the starch grains vary considerably in size (Mes and Menge, 1954).

**Stolon Initiation and Development**

For potato plants raised from seed tubers or stem cuttings, stolon growth may be initiated soon after elongation of the main stem commences. The initiation of stolon growth can occur even before the leafy shoot has emerged (Ewing and Struik, 1992), indicating that it does not depend on signals from the shoot.

Stolons form more readily in darkness and moist conditions than in light and a dry atmosphere (Kumar and Wareing, 1972). On potato plants grown from seed tubers, stolons develop first at the most basal node of the sprout and later at higher nodes (Plaisted, 1957; Cutter, 1992). About half of the stolons form at the most basal node and roughly 10 % of the remaining stolons form at each of the next four higher nodes (Wurr, 1977). The pattern of stolon formation in the three cultivars investigated by

Wurr (1977) were much more uniform than the patterns of tuberization. Under normal conditions, the later a stolon was initiated, the shorter the interval before tuber initiation became dominant (Vreugdenhil and Struik, 1989).

Stolon growth can occur over a wide range of temperatures and daylengths, but the development of stolons into tubers usually requires more specific conditions, for example low temperatures and SD. It is generally accepted that LD favor stolon elongation, while SD result in cessation of stolon growth (Chapman, 1958). However, Ewing (1997) claimed that whether SD increase or decrease stolon growth depended upon how induced the plants were when the treatment was applied. A small decrease in daylength or temperature received by plants grown under very non-inductive conditions (LD, high temperatures) increased stolon production. When stolons were already growing profusely, giving more induction (SD and low temperature) lead to a reduction in stolon elongation and many stolons initiated tuber growth (Ewing, 1997). Hormones were reported to affect the development of stolons. Applying gibberellins and auxin to the stumps of decapitated *Solanum andigena* plants promoted the formation of diagravitropic stolons from lateral buds, when plants had been exposed to SD (Woolley and Wareing, 1972). The developing stolons could be converted into leafy shoots by exposure to light (Dean, 1994). High temperature during growth might also cause stolons to migrate to the surface and became shoots (Struik *et al.*, 1989a; Dean, 1994). Hormones affected the development of stolons. Cytokinin could convert stolons into leafy shoots (Kumar and Wareing, 1972). Gibberellins, in the presence of auxin stimulated stolon elongation, and a high ratio of cytokinin to GA promoted leafy shoot development and a low ratio favored stolon formation (Cutter, 1992).

The pattern of stolon elongation normally consists of an initial lag period followed by more rapid rate of extension. The later the time of stolon initiation, the longer the length of the lag phase (Lovell and Booth, 1969). The elongation rates of individual stolons were not constant. Individual stolons may cease growing and remain in this state for days or weeks before recommencing active growth, while other stolons on the same plant continue elongating (Lovell and Booth, 1969). The first initiated stolon may not elongate more rapidly than later initiated stolons and is often not the longest at harvest (Lovell and Booth, 1969).

Branching during stolon elongation is an important event as branching determines the number of stolon nodes and tips, and therefore the number of potential sites for tuberization. Heavily branched stolons resulted in a large number of potential tuber sites (Helder *et al.*, 1993a). Every stolon node has the potential to form a branch. However, the degree of branching of stolons can be quite variable. The first formed stolons at the basal nodes were more likely to branch (Lovell and Booth, 1969; Struik and Van Voorst, 1986). It was reported that stolon branching might be stimulated by LD (Struik *et al.*, 1988b), high temperatures (Struik *et al.*, 1989b), and high GA levels (Struik *et al.*, 1989c). Irradiance might affect branching as well, as 70 % shading to field-grown plants reduced branch stolon number (Wurr *et al.*, 1997).

In addition to the degree of branching, other factors that are highly variable within and between cultivars include the length of the stolon before tuberization and the location of the stolon in respect to other stolons (Ewing and Struik, 1992).

**Tuber Initiation**

The earliest morphological change during tuberization is the swelling at the subapical zone of the stolon (Artschwager, 1924; Plaisted, 1957; Xu *et al.*, 1998a). In both *in vitro* and *in vivo* conditions, the first site of swelling is often the eighth internode counted from the apical one (Cutter, 1992). It is generally assumed that the longitudinal growth of stolons stops as soon as the swelling of the stolon tip starts (Cutter, 1992; Peterson *et al.*, 1985; Vreugdenhil and Struik, 1989).

Generally, tuberization occurs first on stolons that develop closest to the seed piece (Cutter, 1992) or the basal axillary bud of cuttings (Gregory, 1956). Initiation of further tubers is not synchronous within the plant. Some stolon branches may have commenced tuberization while other branches on the same stolon continue to elongate (Helder *et al.*, 1993a). The proportion of stolons that tuberize is also highly variable (Moorby, 1967; Wurr, 1977; Cother and Cullis, 1985), and the first formed stolons are not always the first to tuberize (Helder *et al.*, 1993a). There is usually little correlation between the time of tuber initiation and the final size (Ewing and Struik, 1992) and the first formed tubers do not necessarily attain the largest size (Wurr,

1977; Struik and Van Voorst, 1986; Struik *et al.*, 1988a). Thus the fate of the individual tuber initial is not predictable.

Wurr (1977), Clark (1921) and Gray (1973) noticed a tendency for smaller tubers on the upper stolons and larger tubers on the lower nodes. However, Krijthe (1955) and Cother and Cullis (1985) observed that most stolons and the largest tubers were generally produced at the third to the fifth node from the base of the stem.

**Tuber Growth**

After tuber initiation, there was a 2 to 3 fold increase in the assimilation of $^{14}CO_2$ and the proportion of assimilates exported from the leaves was doubled, with most of them moved to tubers (Moorby, 1968). The increased translocation of assimilates into tubers resulted in rapid tuber growth. The rate of tuber growth was exponential for the first 2 – 3 weeks and then became almost linear (Mares *et al.*, 1985).

The rate of tuber growth varied between plants and between tubers within a plant (Mares *et al.*, 1985; Schnieders *et al.*, 1988). In the same plant, some of the tubers may be resorbed, some remain small until plant maturity, and others grow to variable sizes (Ewing, 1997). As some tuber initials are resorbed and some remain small until haulm maturity, the number of the harvestable potato tubers is always lower than the number of tuber initials.

Tuber growth is affected by environment, water availability and fertility. The optimal temperature for tuber growth is 15 - 22 °C and when temperature rose to 27 °C, tuber dry weight and tuber number decreased (Dean, 1994). Water stress was shown to be detrimental to tuber growth, and water stress is known to decrease tuber yield and quality. Tuber growth ceased when leaf water potential dropped to –0.4 to –0.5 MPa (Gandar and Tanner, 1976).

**Whole Plant Morphological Change During Tuberization**

The morphological changes at the stolon tip during tuberization coincided with significant changes in other parts of the plant. After tuber initiation, leaves become

large and thinner, and they assume a less acute angle with the stem. Axillary branching is reduced, flower bud abscision is increased, rooting is inhibited, and maturation and senescence are accelerated (Ewing, 1990). The decrease in leaf thickness enables more efficient photosynthesis per unit leaf dry weight (Ewing and Struik, 1992).

The correlations between different plant parts and tuberization have received little attention. In one study, Helder *et al.* (1993a) monitored the stolon characteristics up to the point of swelling, but did not find any clear correlation between swelling and branching order, stolon and stolon branch age, longitudinal growth rates of stolons and stolon branches, and the attachment of the stolon to the main stem.

## Developmental Stages

The growth and development characteristics of crop plants vary during the life of the crop. For most agricultural species, including potatoes, specific agronomic practices are required at different stages in crop growth achieve high yields of good product quality. A set of standard description to divide plant growth into distinctive developmental stages is required to facilitate the implementation of agronomic practices at the approximate time during crop growth. Plant developmental scales may be used by all those involved in production of the crop, particularly for timing in relation to cultural practices, harvesting operations and application of agrochemicals (Knott, 1987).

For potato crops, a number of different growth stages have been proposed (Griess, 1989; Griess and Moll, 1988; Jefferies and Lawson, 1991). They describe the development of potato plants from seed germination or seedling emergence to senescence. For example, Jefferies and Lawson (1991) recommended seven principal stages, including seed germination and seedling emergence, tuber dormancy, tuber sprouting, emergence and shoot expansion, flowering, tuber development and senescence. These scales are sufficient to direct agronomic practices but lack the detail required to separate events occurring in a narrow range of the developmental sequence, such as that from stolon elongation to tuber initation.

For studying potato tuber initiation, the most cited developmental stages were: (1) stolon induction and initiation, (2) stolon growth including the elongation and branching of the stolon, (3) cessation of longitudinal growth of the stolon, (4) tuber induction and initiation (Vreugdenhil and Struik, 1989). When studying the exomorphological and histological changes occurring during *in vitro* tuberization, Melchiorre *et al.* (1997) distinguished four distinct stages in the microtuberization process: (1) development of a thin and cylindrical stolon, (2) curvature and enlargement of the subapical region of stolon, (3) tuber filling as a consequence of the inhibition of the stem elongation and the proliferation of storage tissues, and (4) tuber maturation. This standard includes all major steps in tuber formation and growth reported from previous studies. A similar scale was defined by Ulloa *et al.* (1997), where the four stages were: (1) stolon elongation, (2) subapical enlargement of the stolon, (3) tuber swelling and (4) tuber maturation.

The definition of plant developmental stages at a plant or crop level is more complicated than at the individual stolon level, as the events related to tuberization are asynchronous. Not all potential tuber sites are similarly and simultaneously directed towards the formation of tubers. Hence the definition of a developmental stage at plant level must reflect the stage when a defined proportion of the stolons/tubers reach the particular stage. For example, tuber initiation was defined as 'the time at which a tuberous swelling reaches twice the diameter of the substanding stolon on 80 % of the mainstems or in the absence of stolons when sessile tuberous swellings are 2 mm in diameter in 90 % of tubers' (Jefferies and Lawson, 1991). Similarily, the definition of a developmental stage at crop level must refer to the stage that the majority of the plants are in. The proportion of plants at or beyond a particular stage of development may be used to define the stage. For example, for bean crop management it is often necessary to describe the crop either as 'a proportion at stage...' or 'at a range of stages ... to ...' (Knott, 1987; 1990). Demagante and Vander Zaag (1988) defined the timing of tuber initiation in field potato as '50 % tuberization', which was the period from planting to the day when 50 % of the sampled plants had tubers.

Despite the absence of defined developmental stages for investigation of tuber initiation and early development at the whole plant level in hydroponics, the

principles associated with such scales and the stages used for other purposes in the potato crop have been outlined in sufficient detail in the literature to propose a series of stages. The stages must be defined at the organ (stolon/tuber), plant and crop levels in order to assess the effects of treatment on rate, timing and uniformity of tuber initiation within experimental crops. Confirmation that morphological changes occurring during tuberization in hydroponics are the same as those described in other production systems is required so that key events can be selected to define transition between stages.

# CHAPTER 3A

# POTATO MORPHOLOGY AND DEVELOPMENTAL STAGES

## OUTLINE

***The identification and definition of developmental stages in tuber formation in hydroponically grown plants is a key step in quantifying plant responses to experimental treatments. In this chapter morphological descriptions of plant growth in the hydroponic system are presented and compared with published morphological descriptions to confirm the similarity of the timing of key events in tuber formation, rate of stolon and tuber development and relationships between different developmental processes. The morphological descriptions from the present study were combined with previously published potato developmental scales to develop a system applicable to the study of tuber formation in hydroponically grown plants. The developmental scales were defined at the single stolon/tuber level, the whole plant level and the crop level for multiple plants in a single hydroponic tray. The applicability of the scales are discussed.***

## INTRODUCTION

The aim of this project was to study potato tuberization at the whole plant level in hydroponics. In order to quantify the effects of a range of treatments applied in this study, the growth of hydroponically-grown potato plants had to be separated into discrete developmental stages. While a number of developmental scales for potato growth studies have been published, none have been applied to the quantification of tuber formation in hydroponic systems.

The developmental changes occurring at the single stolon/tuber level have been divided into four stages: stolon induction and growth, cessation of stolon longitudinal growth, tuber induction and tuber intiation (Vreugdenhil and Struik, 1989). The asynchronous pattern of stolon and tuber development complicates the quantification of tuberization of the whole plant level, as different stolons on an individual plant may develop at different rates (Helder *et al.*, 1993a) and timing of different key events may overlap within an individual plant (Vreugdenhil and Struik, 1989).

Plant developmental scales applicable at the crop level are widely used in agricultural practice and in research, particularly for timing of cultural practices, harvesting operations and application of agrochemicals (Knott, 1987). Jefferies and Lawson (1991) split the crop lifecycle of field-grown potato from seed germination to senescence into seven principal developmental stages: seed germination and emergence, tuber dormancy, tuber sprouting, emergence and shoot expansion, flowering, tuber development, and senescence. Recommendations for crop management practices such as irrigation and fertilization have been based on these developmental stages. This scale is valuable in many agronomic studies but is less useful for studies of specific sections of the lifecycle, such as tuberization.

Desirable features on a scale of development include that the characteristics defining each stage are clear and easy to identify, the characteristics occur under all conditions promoting the developmental pathway, and the characteristics exist for long enough for observation and if required the application of experimental treatments. Development of an useful scale is thus dependant on detailed morphological descriptions.

In order to establish a developmental scale for hydroponically grown potato plants, the characteristic morphological features of potato plants during tuberization in hydroponics were studied. Comparision was made to published data to assess if the timing of key events in tuber formation, rate of stolon and tuber development and relationships between different developmental processes differed. Based on the morphological descriptions obtained in this study and published descriptions of

potato development, a scale of plant development during tuberization in hydroponics was established.

# METHODS AND MATERIALS

Morphological changes during development from the transplanting of tissue cultured plantlets to tuber harvesting were recorded in a total of 5 experiments. These experiments were carried in May-June, July-August, September–October in 2000, December-January 2000-2001 and March-April 2001. All experiments were conducted using the hydroponic system and under the glasshouse conditions described in Chapter 2.

Development of stolons and tubers within the hydroponic trays were observed daily. The date of initiation of the first stolon was recorded. After initiation of the first stolon, the colour, structure and size of stolons, number and nodal origin of primary and branch stolons, and stolon length were recorded every 2 or 3 days in all experiments except the May-June trial. The number of swelling tips and tubers, the location of the swelling tips and tubers, the diameter of swelling tips and tubers and any abnormal tuber development were recorded. When most plants started to develop swelling tips, the height and number of shoots per plant and the maximum leaf length and stolon length were recorded. Crops were harvested when all plants had tubers greater than two centimeter in diameter. The maximum stolon length, stolon diameter and tuber appearance were recorded.

Stolon length was measured using a compass and a ruler. The distance between the stolon tip and the node from which the stolon emerged was copied using a compass and the distance between the two legs of the compass was then measured using a ruler. The measurement of shoot height, leaf length and the maximum stolon length at harvesting time was undertaken using a ruler. The diameter of stolons and tubers was measured using vernier calipers.

Total length of stolon and total number of swelling tips and tubers per plant from nine plants per tray were recorded as one replicate.

# RESULTS

## Description of Stolon, Tuber and Shoot Morphology

### Stolon Morphology and Development

Stolons developed from the basal nodes of the shoot system within the hydroponic trays and possessed the characteristic nodes, internodes and small scale-leaves of potato stolons (Plate 1A). A number of variations in stolon morphology were observed. Stolon tips normally deviated slightly from the direction of stolon growth, but under some conditions stolon tips pointed 180° away from the direction of stolon growth (Plate 2). The resultant hooking of the stolon tip has been widely described (Artschwager, 1924; Booth, 1963; Cutter, 1992). Leaf primordia at the stolon tip were generally less than 5 mm in length, but in summer crops large leaf primordia were often found at the apical tip of the stolon and scale leaves greater than 5 mm in length developed on the stolons (Plate 3). More than half of the stolons developing in summer grown crops formed leafy shoots. The transition from stolon to leafy shoot involved a series of changes. These included change in colour from white to green, in direction of growth from horizontal to vertical, and expansion in scale-leaf size at nodes near the stolon apex and at the apex itself. Stolons with visible leaf development at the apex also tended to be hooked and these stolons were more likely to elongate for long periods without initiating tubers. Hooked stolon tips were observed on stolons with small scale-leaves. Swelling tips were almost always observed developing from non-hooked stolon tips (Plate 1, 4).

Stolon thickness, length and distance between nodes were highly variable in hydroponically grown plants. Differences between seasons in mean size of stolons and variability between stolons occurred both through differences in timing and rates of stolon elongation and differences in timing of tuber initiation. The longest individual stolon length at harvest in winter (May-June) was 13 cm; and the longest stolon in summer (December-January) reached 94 cm; while in autumn (March-April) and spring (July-August and September-October) the longest stolons were between 42 and 50 cm (Table 2). Stolon internode length was also greater in summer grown plants than that of other seasons. Stolon growth in winter was more

uniform, with most stolons reaching a maximun length of 5 cm before tuber initiation, compared to between 22 and 32 cm in other seasons (Table 2). Stolon diameter varied greatly (Plate 5 and Table 2), between approximately 1 and 3 mm in autumn, winter and spring grown plants. The maximum stolon thickness was recorded in summer, and the greatest variability in stolon size also occurred in summer. The maximum stolon diameter in summer was 8 mm while the minimum diameter was 1 mm (Table 2). Stolon diameter generally increased with stolon length, with a clear gradation in stolon diameter evident in longer stolons on summer grown plants (Plate 6). The number (mean ± standard error) of the primary stolons per plant increased from winter (May-June) to summer (December-January), 2.8 ± 1.4 and 9.1 ± 3.1 respectively, while the number of branches showed a similar trend, increasing from 0. 6 ± 1.3 per plant in winter (May-June) to 9.6 ± 7.1 per plant in summer (December-January) of (Table 2).

Branching and adventitious root development were observed from nodes on elongating stolons in all seasons. Branching generally occurred firstly from the basal 1 - 2 nodes of the primary stolon (Plate 9) and these branch stolons were normally longer than later formed branches. The growth rate of stolons was highly variable and was not linked to stolon position. After branches had initiated, both the main and branch stolons were able to grow rapidly. The growth rate of the primary stolon was not always faster than that of its branches. The growth rate of branches originating from the same stolon were often significantly different.

There was a lag phase of approximately 3 - 5 days between planting and initiation of stolon growth, during which no visible growth of the plant occurred (Plate 7 and Plate 8). Stolon growth could be initiated from any nodes held in darkness within the hydroponic trays. However, the first stolon initiated was normally at the basal node or the second from basal node on the plant (Plate 9). Stolon initiation occurred progressively at higher nodes on the plantlet. The direction and the location of stolon growth when first initiated determined its fate. During the first few days after initiation, stolons that grew upright or failed to touch the capillary matting in the hydroponic trays were less likely to develop normally than stolons in contact with the hydroponic solution. After initiation, stolons that contacted the matting or

developed roots into the matting normally showed vigorous growth and initiated tubers. The longest and most rapidly growing stolons were normally those close to the matting when they first initiated.

Developing stolons in the hydroponic system were observed to have a number of different fates. The most common occurrence for the stolon tips was to swell to form a tuber. Stolons were also observed to cease elongating and not to develop further. Under some conditions these stolons developed necrotic lesions near the stolon tip and died. This necrosis appeared to be a physiological disorder rather than the result of pathogen attack. Under other conditions, stolon tips could initiate formation of leafy shoots. The occurrence of the different developmental patterns varied with season.

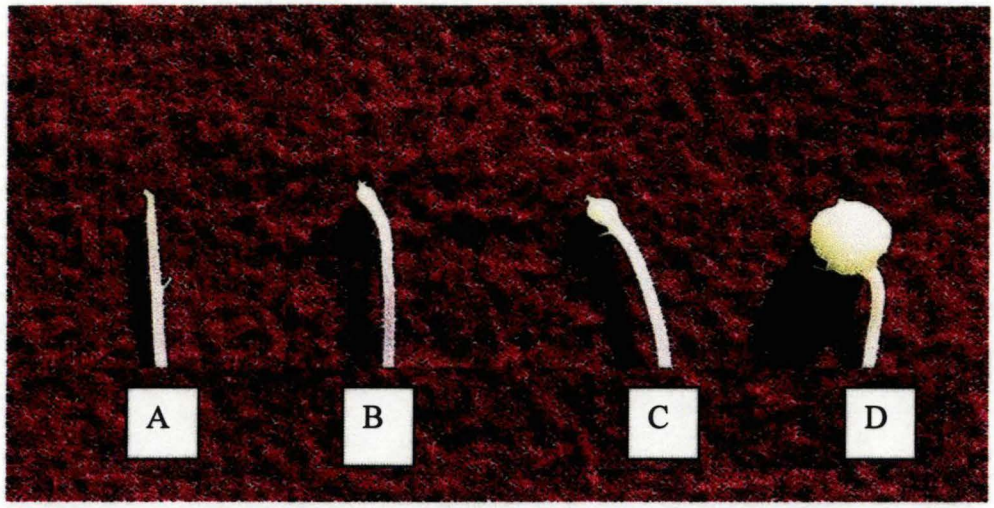


**Plate 1.** The main morphological features of elongating stolon (A); initiation of swelling tip (B); swelling tip (C) and tuber (D) from the July-August (winter) crop.

**Plate 2.** Hooked stolon in the December-January (summer) crop.

**Plate 3.** Stolon developed into shoot in the December-January (summer) crop.

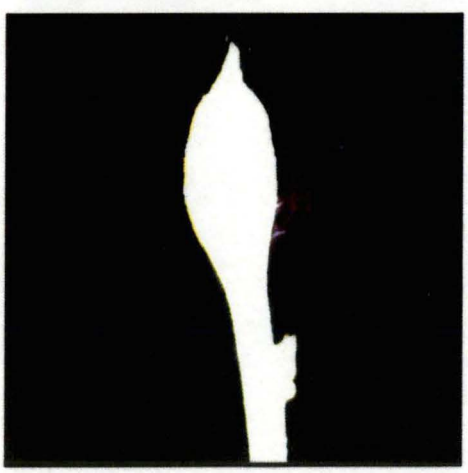

**Plate 4.** Non-hooking swelling tip from the September-October (spring) crop.

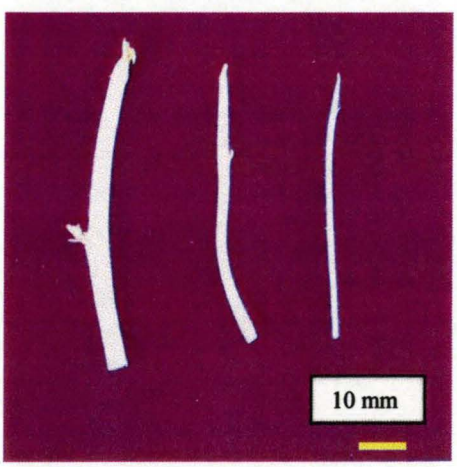


**Plate 5.** Stolon diameter varied from 0.15 to 6.0 mm from the December-January (summer) crop.

**Plate 6.** Stolon diameter increases gradually as it elongates in the summer crop.

**Plate 7.** Tissue-cultured plantlet at the time of planting.

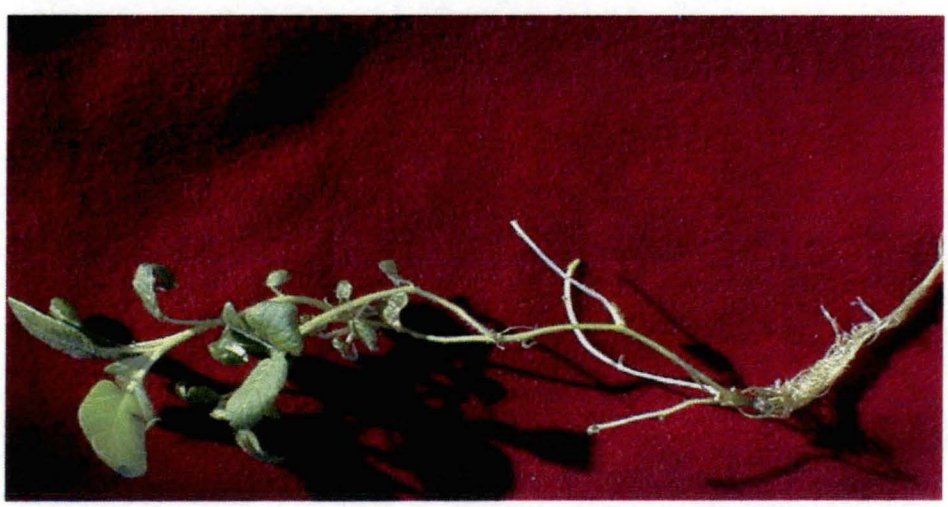

**Plate 8.** Plant of stage 2 (stolon elongation) from the May-June (late autumn-early winter) crop.

**Plate 9.** Plant of stage 3 (swelling) from the May-June (late autumn-early winter) crop.

**Plate 10.** Plant of stage 4 (setting) from the May-June (late autumn-early winter) crop.

**Plate 11.** Plant of stage 5 (bulking) from the May-June (late autumn-early winter) crop.

**Table 2.** Morphological description of potato growth in hydroponics.

| | Morphological description | | | | |
|---|---|---|---|---|---|
| **Experiment period** | **Mar-Apr** | **May-Jun** | **Jul-Aug** | **Sep-Oct** | **Dec-Jan** |
| **Range of shoot length at swelling (cm)** | 10 - 30 | 10 - 15 | 10 - 30 | 10 - 30 | 20 - 30 |
| **Range of leaf length at swelling (cm)** | 2 - 10 | 2 - 4 | 2 - 10 | 2 - 10 | max 21 |
| **Range of shoot number per plant** | 2 - 4 | 1 - 3 | 1 - 3 | 2 - 4 | 5 - 9 |
| **Node of the first stolon initiated** | basal 1-2 nodes | basal 1-2 nodes | basal 1-2 nodes | basal 1-2 nodes | basal 1-2 nodes |
| **Number of primary stolons (N = 9)** | 5.0 ± 1.4 | 2.8 ± 1.4 | 4.7 ± 1.4 | 6.9 ± 1.8 | 9.1 ± 3.1 |
| **Stolon thickness (mm)** | 1 - 3 | 1 - 3 | 1 - 3 | 1 - 3 | 1 - 8 |
| **Maximum stolon length at swelling (cm)** | 32 | 5 | 22 | 28 | 27 |
| **Maximum stolon length at harvest (cm)** | 50 | 13 | 42 | 42 | 94 |
| **Number of stolon branches per plant (N = 9)** | 8.7 ± 3.6 | 0.6 ± 1.3 | 2.4 ± 2.8 | 3.8 ± 1.9 | 9.6 ± 7.1 |
| **Proportion of stolons with pronounced hooking** | medium | very low | low | medium | high |
| **Leafy stolons** | few | no | no | few | many |
| **Colour changes prior to swell** | yes | yes | yes | yes | yes |
| **Swelling from hook stolon** | few | few | few | few | few |

## Tuber Initiation

The initial swelling of stolons prior to tuber formation occurred in the subapical zone of the stolon, approximately 1 to 3 mm behind the stolon tip. The length of the zone where swelling occurred was usually 3 to 5 mm, depending on stolon thickness, with a longer swelling zone on thicker stolons. Some stolons were found to expand over an extended zone behind the apex. In these stolons, swelling was observed to occur in a zone up to 20 mm long. This swelling did not always result in tuber formation. However, if tuber formation proceeded from these stolons, the swelling became more pronounced in the subapical zone.

One to three days prior to any visible swelling of a stolon, a characteristic colour change was often observed. The brightness and intensity of the white coloration of stolon tips increased, giving the surface a smooth appearance. This change was observed prior to swelling in stolons in each season. In addition to the characteristic change in colour, it was also observed that swelling was more likely in rapidly elongating stolons rather than in slowly elongating stolons or stolons that had ceased elongation.

## Tuber Morphology and Development

Tuber formation was observed to occur at the tips of primary and branch stolons and at nodes on stolons. The earliest swelling tips were generally observed from the stolons growing from the basal 1 to 2 nodes (Plate 9). The stolons from higher nodes were able to swell later. Aerial tubers were occassionally observed in leaf axils in summer grown plants (December-January).

The fate of newly initiated tubers was not predictable. Some tuber initials expanded rapidly while others expanded quite slowly. The rapidly expanding tubers were normally a light brown to white colour, while slowly expanding tubers were a darker brown colour. Most tubers continued to expand after initiation, but there were a small percentage of tubers that failed to expand further. Resorption was rarely observed in the hydroponic system. Resorption was only observed in summer plants, and only occurred in tubers initiated late in crop development in crops grown for a duration of 80 days or longer.

The colour of tubers changed from white to brown as tuber size increased. Tuber shape varied with growth season. In winter, tuber shape was more uniform as most tubers were a spherical or elongated oval shape, while in summer more irregularly shaped tubers were observed.

Growth rate varied greatly between tubers on the same plant and it was impossible to predict which tuber would undergo rapid growth. Growth rate was not linked to order or timing of tuber initiation. The earliest swollen tip did not always develop into the biggest tuber. In the presence of several large tubers in one plant, other small tubers rarely grew rapidly or attained a large size. However, excising large tubers lead to rapid growth in the remaining small tubers.

Tuber regrowth and the development of chain tubers were observed occasionally. Tubers that ceased growth could regrow from any eye of the original tuber. The skin of the regrowing portion of the tuber was white and the old portion tended to be a dark brown colour. Tubers that stopped growth were also able to recommence stolon growth from the apical end or other eyes on the tuber. These stolons could elongate or form new tubers after a short period of stolon growth, resulting in chain tuber development. Tuber regrowth was generally observed in autumn, spring and summer plants, while chain tubers were only observed in summer plants.

**Shoot Morphology**

Large differences in the plant size, leaf size and shoot number were observed between crops grown in different seasons. In winter plant size was relatively small, with shoot height between 10 and 15 cm when tuber initiation commenced while in summer shoot height was between 20 and 30 cm when tuber initiation occurred (Table 2). Leaf length in winter plants was approximately 2 to 4 cm while in summer the maximum leaf length reached 21 cm (Table 2). Less shoots were formed in winter grown plants than in summer grown plants. Shoot number per plant varied between 1 to 3 and 5 to 9 in winter and summer respectively.

# Developmental Stages

### Defining Stages of Development

The characteristic developmental stages for hydroponically grown potato plants were developed based on published scales in the literature (Vreugdenhil and Struik, 1989; Melchiorre *et al.*, 1997; Ulloa *et al.*, 1997). The stages included plant establishment (prior to stolon initiation), stolon elongation, swelling, tuber setting and tuber bulking. This set of stages did not include some of characteristic stages reported in literature, such as stolon curvature, cessation of longitudinal stolon growth and tuber induction. Stolon curvature was not a common phenomenon within hydroponics. Cessation of stolon elongation was observed both before and after swelling and tuber setting and was therefore not a useful morphological change to include in either organ or plant level developmental stages. Tuber induction cannot be observed, as it is an internal physiological change rather than a morphological phenomenon.

The stolon level scale for hydroponically grown plants was defined in Table 3.

**Table 3.** Stolon level developmental stages of hydroponically grown potato plants

| Stage code | Stage name | Morphological keys |
|---|---|---|
| 1 | Stolon initiation | Commences at planting and ends when stolon is 1.0 cm in length |
| 2 | Stolon elongation | Commences when stolon is 1.0 cm in length and ends when stolon swelling commences |
| 3 | Swelling | Commences at initiation of swelling and ends when diameter of the subapical zone is 2 times of stolon diameter |
| 4 | Tuber setting | Commences when tuber is > 2 times of stolon diameter and ends when tuber diameter is 1.0 cm |
| 5 | Tuber bulking | Commences at tuber diameter is 1.0 cm |

Based on the stolon level scale, the plant level scale was defined in Table 4.

**Table 4.** Plant level developmental stages of hydroponically grown potato plants

| Stage code | Stage name | Morphological key |
|---|---|---|
| 1 | Plant establishment | Commences at planting and ends when plant has developed at least one stolon of 1.0 cm length (Plate 7) |
| 2 | Stolon elongation | Commences when the longest stolon reaches 1cm and ends when one swelling stolon tip appears (Plate 8) |
| 3 | Swelling | Commemces when one visible swelling tip appears and ends when the first tuber diameter is 2 times of stolon (Plate 9) |
| 4 | Tuber setting | Commences when the first tuber diameter is > 2 times of stolon ends when the first tuber diameter is 1.0 cm (Plate 10) |
| 5 | Tuber bulking | Commences when the first tuber reaching a size of greater than 1cm diameter to later (Plate 11). |

Crop level stages were defined based on the percentage of plants that had reached each stage (Table 5). This method has been widely used in defining developmental stages in other crops (Knott, 1987, 1990; Jefferies and Lawson, 1991).

The developmental stages were used to describe the seasonal pattern in crop development in hydroponics. Plants were examined regularly and timing of key characteristics at the stolon was recorded for each plant. The data were tabulated to determine timing of developmental events at the plant and crop level. An example on data for a single crop was shown in Table 6.

**Table 5.** Crop level developmental stages of hydroponically grown potato plants

| Stage code | Stage name | Key |
|---|---|---|
| 1 | Plant establishment | Commences at the day of planting and ends when 7 out of the 9 plants developed at least one stolon of at least 1.0 cm in length |
| 2 | Stolon elongation | Commences when 7 out of 9 plants developed at least one stolon of at least 1.0 cm and ends when 7 out of 9 plants developed at least one swelling tip |
| 3 | Swelling | Commences when 7 out of 9 plants have developed at least one swelling tip and ends when 7 out of 9 plants have at least one swelling tip with diameter > 2 times of stolon |
| 4 | Tuber setting | Commences when 7 out of 9 plants have developed at least one swelling tip with diameter > 2 times of stolon ends when 7 out of 9 plants have at least one tuber of 1.0 cm diameter |
| 5 | Tuber bulking | Commences when 7 out of 9 plants have at least one tuber with a diameter of 1.0 cm |

Crop level stages were calculated based on the morphological description of stolons at the plant level (Table 6). At day 11, an average of 5 out of 9 plants had one or more stolons at stage 2 (stolon elongation), but plants were not in stage 2 at the crop level. Crop level stage 2 was first recorded at day 14 when an average of 7 out of 9 plants were at stage 2. At day 23, an average of 5.7 out of 9 plants had one or more stolons at stage 3 (swelling), but at the crop level stage 3 was not recorded until day 25 when 7 out of 9 plants were at stage 3. At day 28 after planting an average of 7.3 out of 9 plants have reached stage 4 (tuber setting), hence the setting stage of the crop level was recorded as day 28 after planting. Crop level stage 5 (tuber bulking) started from day 32 after planting when an average of 7 out of 9 plants were at stage 5.

**Table 6.** Determining of developmental stage from plant and crop levels (Results from the July-August crop 2000).

| | Number of plants (out of 9) with the key characteristics | | | | | | | | | | | | | | | | Crop level developmental stage | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stolon > 1.0 cm | | | | Swelling tip | | | | Tip > 2 x stolon diameter | | | | Tuber diameter > 1.0 cm | | | | Stage code | Stage duration (days) |
| DAP | Tray 1 | Tray 2 | Tray 3 | ave | Tray 1 | Tray 2 | Tray 3 | ave | Tray 1 | Tray 2 | Tray 3 | ave | Tray 1 | Tray 2 | Tray 3 | ave | | |
| **7** | 0 | 0 | 0 | 0 | | | | | | | | | | | | | 1 | |
| **9** | 3 | 0 | 2 | 1.7 | | | | | | | | | | | | | 1 | |
| **11** | 6 | 3 | 6 | 5 | | | | | | | | | | | | | 1 | 14 |
| **14** | 7 | 6 | 8 | 7 | | | | | | | | | | | | | 2 | |
| **16** | 8 | 8 | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | 2 | |
| **21** | 9 | 9 | 9 | 9 | 4 | 3 | 4 | 3.7 | 3 | 2 | 3 | 2.7 | | | | | 2 | |
| **23** | | | | | 6 | 5 | 6 | 5.7 | 3 | 5 | 4 | 4 | | | | | 2 | 11 |
| **25** | | | | | 7 | 6 | 8 | 7 | 5 | 4 | 5 | 4.7 | 0 | 0 | 0 | 0 | 3 | 3 |
| **28** | | | | | 8 | 9 | 9 | 8.7 | 7 | 8 | 7 | 7.3 | 4 | 3 | 4 | 3.7 | 4 | |
| **30** | | | | | 9 | 9 | 9 | 9 | 8 | 9 | 9 | 8.7 | 5 | 4 | 6 | 5 | 4 | 4 |
| **32** | | | | | | | | | 9 | 9 | 9 | 9 | 7 | 7 | 7 | 7 | 5 | |
| **34** | | | | | | | | | | | | | 8 | 9 | 8 | 8.3 | 5 | |

One of the important features of a usable developmental stage was that every stage should last long enough for observation and application of experimental treatments if required. The duration of each stage was calculated for the July-August crop (Table 6) and all other crops was recorded in the morphological study. While the duration of each stage varied with season, each lasted for three or more days (Table 7), indicating that the key characterisitcs used in defining the stage allowed adequate temporal seperation of the stages.

**Table 7.** Duration of each stage (days) at the crop level in different growth seasons.

| Stage | Mar-Apr | May-Jun | Jul-Aug | Sep-Oct | Dec-Jan |
|---|---|---|---|---|---|
| **1** | 14 | 16 | 14 | 14 | 15 |
| **2** | 9 | 4 | 11 | 13 | 30 |
| **3** | 4 | 5 | 3 | 4 | 4 |
| **4** | 4 | 8 | 4 | 8 | 12 |

# DISCUSSION

The morphological characteristics and growth patterns associated with stolon and tuber development in hydroponics were consistent with those reported in the literature for potato plants grown in other productive systems. The only notable difference observed was that formation of the hook stage, or bending at the subapical region prior to swelling (Booth, 1963; Cutter, 1992), did not occur frequently in hydroponically grown plants. The hook stage reported in field grown plants was only observed in summer crops in hydroponics in this research, and was often associated with stolon elongation rather than the commencement of swelling of the stolon tip. Very few stolons had any subapical curvature at the commencement of swelling.

The initiation of the first stolon in hydroponics was from the basal nodes of the plantlet and later stolons formed from the higher nodes. This pattern was similar to that of potato plants grown in glass tanks in culture solution (Lovell and Booth, 1969), and that of potato plants grown from seed tubers (Cutter, 1992; Plaisted, 1957). Stolons seldom appeared from nodes that were exposed to light. Stolon growth was also poor when not in contact with the capillary matting in the hydroponic system trays. This was consistent with the conclusion by Kumar and Wareing (1972) who reported that darkness and a moist environment favoured stolon growth over light and dry atmospheres. Leafy stolons were often observed in hydroponics, but these stolons always originated from higher nodes on the plant and were exposed to diffuse light due to the construction of the hydroponic system. Stolons growing in darkness within the hydroponic trays in summer in this research often developed leaves on them or were converted into leafy shoots. This supported the previous conclusion that high temperature has a significant impact on stolon development and promotes shoot development from stolons (Struik *et al.*, 1989b).

Development of stolon branches occurred a short period after the main stolon commenced elongation. Branching could occur from any node on any stolon. The first branch tended to form from the basal node of the primary stolon, but this was

not always the case. This was similar to the observations by Lovell and Booth (1969) and Struik and Van Voorst (1986) that the first formed stolons at the basal nodes were more likely to branch. More branches were recorded from plants grown in summer than in winter suggesting that summer conditions favour stolon branching. Factors such as LD (Struik *et al.*, 1988b), high temperatures (Struik *et al.*, 1989b), high GA levels (Struik *et al.*, 1989c) and high irradiance (Wurr *et al.*, 1997) have been reported to promote branching.

Stolon thickness varied from 1 to 3 mm in plants grown in all seasons except summer. In summer stolon thickness varied from 2 to 8 mm. Comparable variability of stolon thickness, from less than 1 mm up to 4 mm, was shown by Helder *et al.* (1993a).

The pattern of swelling in hydroponics was consistent with that reported in the literature, with the first swelling occurring in the subapical zone of the stolon (Artschwager, 1924; Plaisted, 1957). The distribution of swelling in hydroponics was irregular, with swelling occurring in stolons and stolon branches over several weeks. There was no correlation between timing of swelling in individual stolons and position or age of the stolon. This was similar to published results (Helder *et al.*, 1993a).

The first signs of tuber initiation in intact plants have been reported as characteristic morphological changes associated with swelling in the subapical zone of stolons (Artschwager, 1924; Plaisted, 1957). In this study, it was found that a slight colour change in stolon tips could be used as a parameter for predicting swelling before any swelling was observed. The brightness and intensity of the white coloration of stolon tips increased from 1 to 3 days prior to any visible swelling. This characteristic change was observed in stolons in each season. This indicator facilitated the study of tuber formation in hydroponics.

No crop level developmental scale for potato crops grown in hydroponics had been published prior to this project. The development of a scale was necessary for this study, as the commercial industry partner for this project identified investigation of the effect of nutrient uptake on potato tuber initiation as a key area for research.

Information from the study could be used to increase the efficiency of minituber production through improving timing and rate of nutrient application. The study of nutrient uptake requires separation of plant growth into discrete developmental stages so that measurements were repeatable and comparable at each stage.

Five developmental stages describing hydroponically grown potato plants from planting of tissue cultured plantlets to tuber bulking at crop level were outlined in this study. Clear and easy to identify morphological characteristics were used to define transition between stages. The characteristics occurred under all growth conditions imposed in the study and were consistent with published observations of morphological characteristics (Kumar and Wareing, 1972; Peterson *et al.*, 1985; Cutter, 1992; Melchiorre *et al.*, 1997). Crop level developmental stages were based on plant level stages and the characteristics selected for the plant level scale were based on organ level stages. Length and number of stolons, and number and size of swelling tips/tubers were the basic parameters used. These characters are clearly and easily recognizable without any special training and equipment. Hence, it is easy to find which stage the plants are in and when a stage starts and ends, if one records the morphological changes frequently.

The stages of development defined in this study had several features that made them suitable for application in the research undertaken in the project. In addition to using characteristics that were easy to recognize and measure, the duration of each plant and crop level stage was at least three days. This enabled measurements to be taken and treatments to be applied at each stage within a reasonable timeframe. At the individual stolon/tuber level, the morphological changes marking transition between stages always occurred during tuber formation and the sequence of changes was always the same. Plants often contained stolons and tubers at different developmental stages but the use of specific numbers of stolons or plants at each developmental stage permitted separation of stages at the plant and crop level.

In defining and validating the stages, differences in duration and uniformity of stages were noted between seasons. Quantitation of these differences demonstrated the potential value of stages in studying tuberization. Application of this approach is presented in the next chapter.

# CHAPTER 3B

# TIMING AND RATE OF STOLON AND TUBER DEVELOPMENT

## OUTLINE

***The main morphological features in hydroponically grown potato plants were recorded in Chapter 3A and criteria were defined to divide plant development from tissue cultured plantlet to tuber bulking into 5 developmental stages. The series of defined developmental stages permits the quantitative comparison of potato development between different growth seasons and the study of correlations between stolon and tuber development. This chapter investigated the timing and rate of stolon and tuber development at both stolon and crop levels in crops grown at different times of the year. The relationship between stolon and tuber development was discussed.***

## INTRODUCTION

Potato tuberization is a process involving a series of morphological and physiological changes during development from stolon to tuber. The timing of the key morphological changes during tuberization, both at stolon and crop level, is important in any study of the physiology of potato tuberization. The timing of morphological changes can be indicative of physiological event, and can also be used in comparative studies assessing the impact of different treatments on tuberization.

While growth stages have been defined for field grown potatoes (Griess, 1987; Griess and Moll, 1988; Jefferies and Lawson, 1991), duration and timing of stages

have not received much research attention. The timing of onset of tuber initiation has been discussed in the literature (Nelson and Hwang, 1975; Manrique *et al.*, 1984), as it is the most important phenological stage in crop development. Nelson and Hwang (1975) grew cultivar Norland in pots in greenhouse and reported that the initiation and early development of tuber stage was between 42 to 49 days after planting. However Manrique *et al.* (1984) recorded that tuber initiation of field grown cultivar Kennebec in winter and summer began at 40 and 55 days after planting respectively.

Initiation of tubers in whole plants grown in modified hydroponic culture has been studied (Krijthe, 1955; Lovell and Booth, 1969; Helder *et al.*, 1993a), but the timing of stolon and tuber development has not been recorded. Quantitative study of the timing of developmental stages in hydroponic crops was therefore undertaken in this study to describe the seasonal variations in tuberization.

# METHODS AND MATERIALS

Timing and rate of stolon and tuber development were calculated in the May-June, July-August, September-October (2000), December-January (2000-2001) and March-April (2001) crops described in Chapter 3A. Plants were monitored regularly after planting and the timing of initiation of stolon growth was determined. After initiation, stolons were marked by placing a small piece of numbered paper (10mm x 10mm) under each stolon, and the length of each stolon and stolon branch was measured using a compass and a ruler in the July-August, September-October (2000), December-January (2000-2001) and March-April (2001) crops. Measurement was every 2 to 3 days from early stolon elongation to swelling, and every 5 to 7 days after swelling. From the occurrence of the first swelling tip on any plant, the number of swelling tips was recorded every 2 to 3 days. As swelling tips developed into tubers, the number of tubers was recorded.

Growth rate and timing of swelling of individual stolons were assessed in the May-June, July-August and September-October crops. Nine stolons were selected at the commencement of the stolon growth stage. The selected stolons were on separate plants. Stolon length was measured at intervals of 2 to 4 days. Measurements ceased for each stolon when the swelling stage (stage 3) was reached.

Daily measurement of stolon length was undertaken in a May-June 2002 crop. Ten stolons, each on separate plants, were selected during the stolon growth stage (stage 2). Only rapidly elongating stolons were selected. Stolon length was recorded at 16:00 daily until 2 to 3 days after swelling was first observed (stage 3). The daily relative growth rate was calculated from the stolon length data.

# RESULTS

## Timing of Key Events (Plant Level)

The earliest stolon initiation occurred between 8 to 9 days after planting for plants grown in July-August, while in other trials from 9 to 12 days after planting (Table 8). The duration of stolon elongation varied with seasons, with the longest stolon elongation in the December-January crop (Fig. 3A). After initiation, stolons elongated rapidly until swelling of the tips occurred. The total length of all stolons on a plant therefore increased rapidly after stolon initiation ccurred but slowed down when the number of swelling tips reached a maximum. Rate of increase in stolon length was slow in the December-January crop until 31 days after planting, but stolon length increased rapidly at about the time when swelling commenced.

**Table 8.** Timing of key events from plant level. All figures are days after planting. Data were not collected daily so some of key events could not be recorded to the nearest day.

| | Mar-Apr | Jul-Aug | Sept-Oct | Dec-Jan |
|---|---|---|---|---|
| **Stolon initiation** | 9 - 11 | 8 - 9 | 9 - 12 | 9 - 11 |
| **Max stolon length** | 26 | 28 | 32 | 38 |
| **Swelling tip occurring** | 21 | 20 | 22 | 35 - 38 |
| **Max number of swelling tips** | 31 | 30 | 29 | 49 |
| **Tuber <1 cm occurring** | 23 | 21 | 23 | 40 - 46 |
| **Max number of tuber < 1 cm** | 31 | 32 | 32 | > 61 |
| **Tuber > 1 cm** | 29 | 25 | 28 | 46 |

Swelling of stolon tips commenced at the time of the early rapid stolon elongation in all crops recorded. Swelling commenced earlier in the September-October, July-August and March-April crops compared to the December-January crop. Swelling commenced at around 22, 20 and 21 days after planting in the September-October, July-August and March-April crops respectively, and between 35 and 38 days after planting in the December-January crop. The number of swelling tips per plant

reached a maximum value between 29 and 31 days after planting in the March-April, July-August and September-October crops, while the peak in number of swelling tips in the December-January crop occurred 49 days after planting (Table 8 and Fig. 3B). The number of swelling tips per plant then decreased as tuber development proceeded.

The time of the first occurrence of tubers less than one centimeter in diameter varied greatly among different growth seasons. Tubers less than one centimeter in diameter were first recorded 21, 23 and 23 days after planting in plants grown in July-August, September-October and March-April respectively, and between 40 and 46 days after planting in the December-January crop. The number of tubers less than one centimeter in diameter increased after the first occurrance until they reached their maximum value 31 to 32 days after planting in the July-August, September-October and March-April crops. In the December-January crop, the number of tubers less than one centimeter in diameter was still increasing when the trial was terminated 61 days after planting (Table 8 and Fig. 3C).

In all cases the first stolon in the crop to commence swelling was also the first to reach the setting stage. This demonstrated that visible swelling was the first morphological indicator of the irreversible commitment to tuber formation.

Tuber bulking was defined as commencing when tuber diameter was greater than 1.0 cm. The time of commencement of the bulking stage varied with the growth season. Tubers greater than 1.0 cm were first recorded 25, 28, 29 and 46 days after planting in the July-August, September-October, March-April and December-January crops respectively (Fig. 3D).

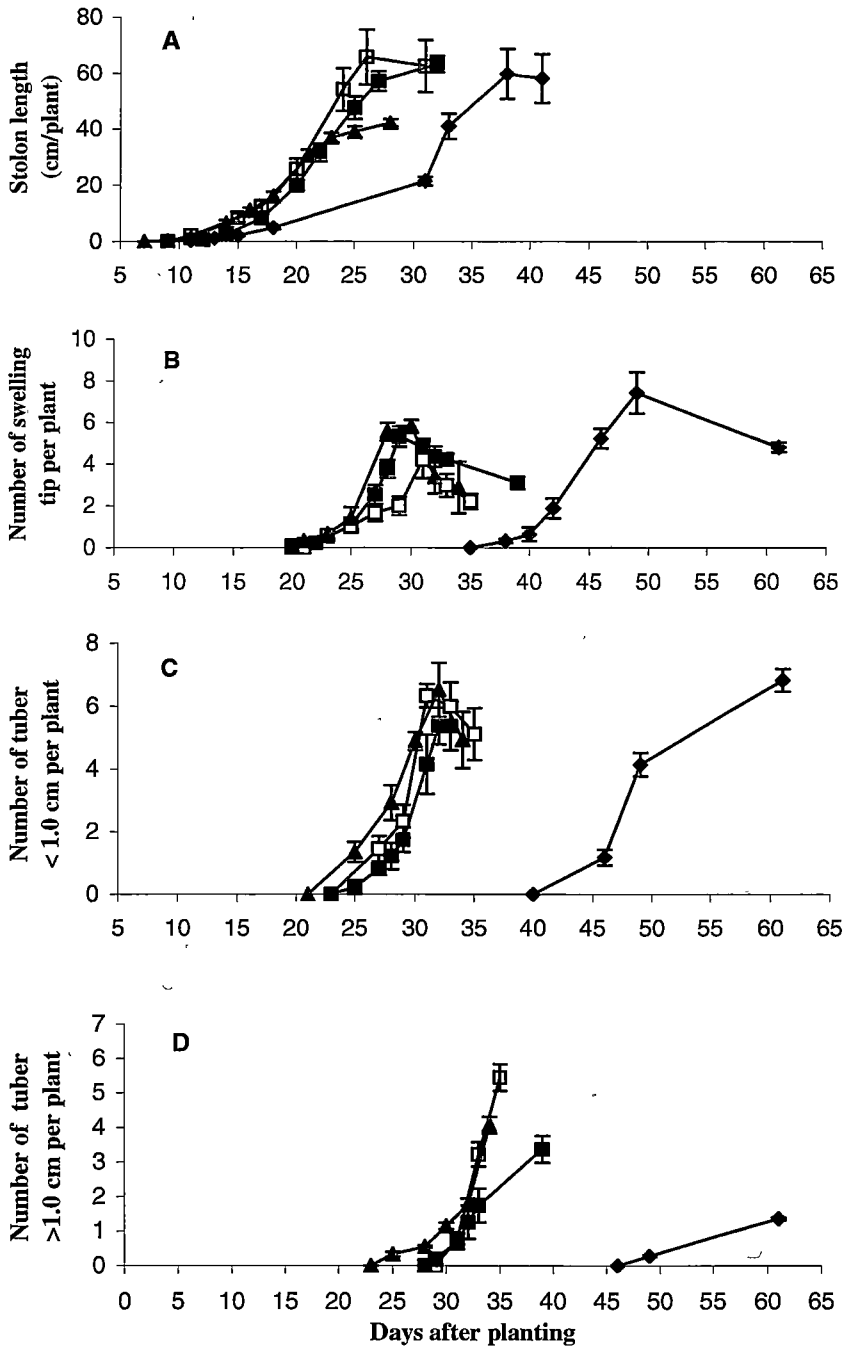


**Figure 3.** Changes with time of stolon length (A), swelling tip number (B), tuber number (<1.0cm) (C) and tuber number (>1.0cm) (D) from plants grown in December-January (◆), September-October (■), July-August (▲) and March-April (□). Each point is the mean of three replicates with standard error bars. Each replicate is a single hydroponic tray containing nine plants.

## Timing of Stages (Crop Level)

Large differences in the timing and duration of stages were recorded between crops grown at different seasons of the year (Table 9). Tuber initiation and development was most rapid in crops grown in winter (May-June, July-August) and was significantly delayed in summer crops (December-January). However, the plant establishment and the swelling phases were relatively uniform. The plant establishment stage lasted 14 to 16 days and swelling stage lasted for 3 to 5 days in all crops.

**Table 9.** Commencement date and duration (number of days after planting in brackets) of crop development stages. Duration of the bulking stage was not recorded. All figures are in days.

| Crop level stage | Mar-Apr | May-Jun | Jul-Aug | Sep-Oct | Dec-Jan |
|---|---|---|---|---|---|
| **Plant establishment** | 1 (14) | 1 (16) | 1 (14) | 1 (14) | 1 (15) |
| **Stolon elongation** | 14 (9) | 16 (4) | 14 (11) | 14 (13) | 15 (30) |
| **Swelling** | 23 (4) | 20 (5) | 25 (3) | 27 (4) | 45 (4) |
| **Setting** | 27 (4) | 25 (8) | 28 (4) | 31 (8) | 49 (12) |
| **Bulking** | 31 | 33 | 32 | 39 | 61 |

The stolon elongation stage commenced at 14, 16, 14, 14, and 15 days after planting in crops grown in March-April, May-June, July-August, September-October and December-January respectively (Table 9). The consistent timing indicated that stolon elongation was capable of commencing as soon as sufficient root growth and other developmental events associated with plant establishment after transplanting into hydroponics were completed. The duration of the stolon elongation stage varied greatly with the season. Duration of the stolon elongation phase was 9, 4, 11, 13 and 30 days for crops grown in March-April, May-June, July-August, September-October and December-January respectively (Table 9).

The swelling stage commenced at 23, 20, 25, 27 and 45 days after planting in crops grown in March-April, May-June, July-August, September-October and December-

January respectively (Table 9). The duration of the crop level swelling stage was relatively uniform, varying from 3 to 5 days across all seasons studied in the project.

The setting stage commenced at 27, 25, 28, 31 and 49 days after planting in crops grown in March-April, May-June, July-August, September-October and December-January respectively (Table 9). The duration of this stage was 4, 8, 4, 8 and 12 days in plants grown in March-April, May-June, July-August, September-October and December-January respectively.

The bulking stage commenced 31, 33, 32, 39 and 61 days after planting in plants grown in March-April, May-June, July-August, September-October and December-January respectively (Table 9).

## Stolon Elongation Rate

Stolon growth rate was low for 6 to 14 days after stolon initiation (Fig. 4). The period of slow stolon growth was followed by a period of relatively rapid growth rate, followed by a rapid decline. The maximum stolon growth rates were 7.14 ± 1.07, 4.81 ± 0.83, 5.90 ± 0.65 and 10.40 ± 3.74 cm/day/plant in March-April, July-August, September-October and December-January respectively. The decline in stolon growth rate generally coincided with plants reaching the tuber setting stage.

The occurrence of the first swelling tip was at approximately the same number of days (20-21) after planting as the maximum stolon elongation rate in the March-April, July-August and September-October crops, while for the December-January crop the first swelling tip was recorded at 36 days after planting, approximately 3 days after the peak in stolon elongation rate (Fig. 4).

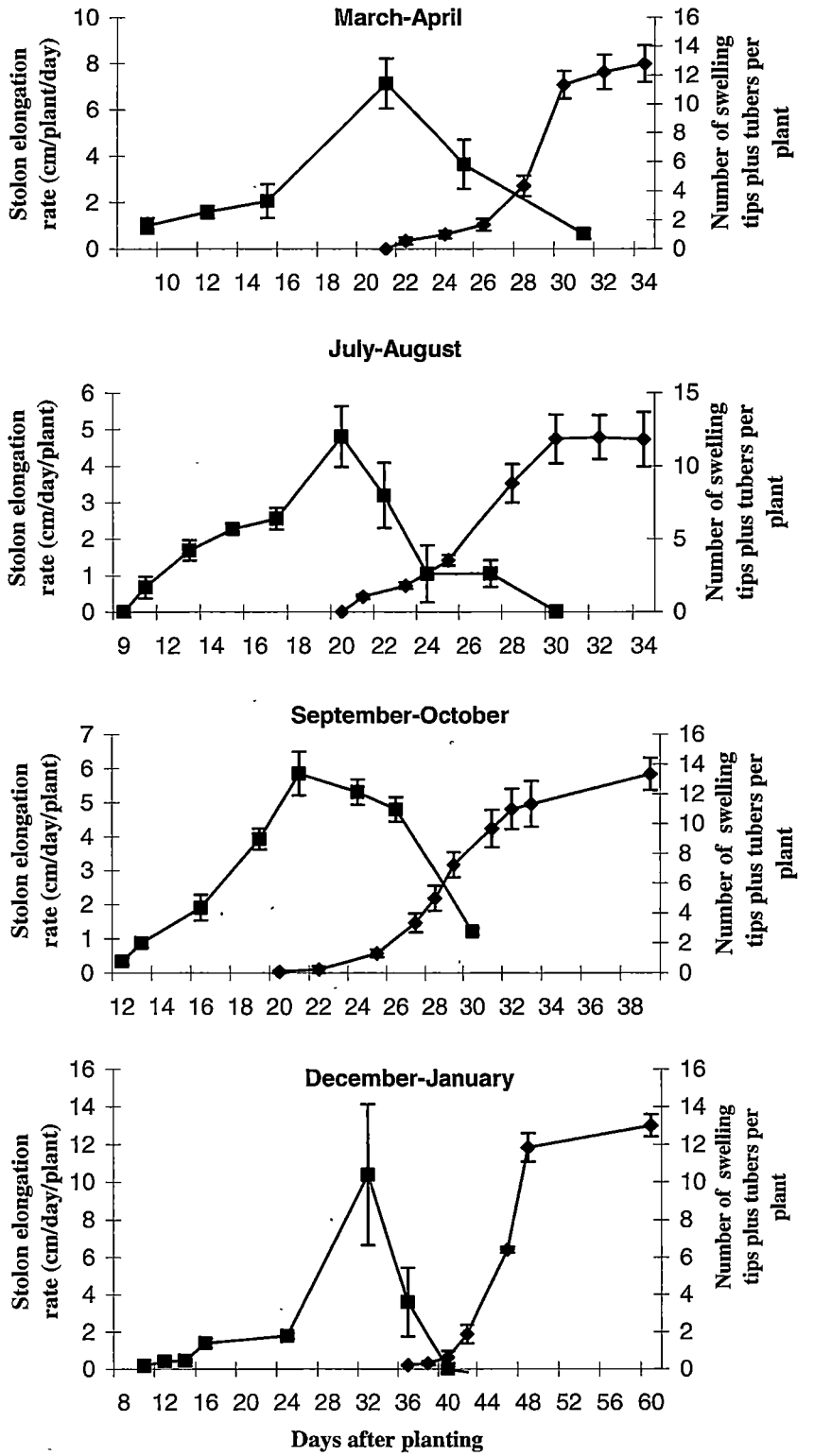


**Figure 4.** Changes in stolon elongation rate (■) and number of tuber initiated (◆) during crop development in different seasons. Each point is the mean of three replicates with standard error bars. Each replicate is a single hydroponic tray containing nine plants.

Growth rate of individual stolons displayed a similar pattern to that of the combined growth rate of all stolons on a plant. Stolon relative growth rates peaked 1 to 3 days prior to swelling and then declined. After swelling, the stolon relative growth rates decreased toward to zero (Fig. 5).

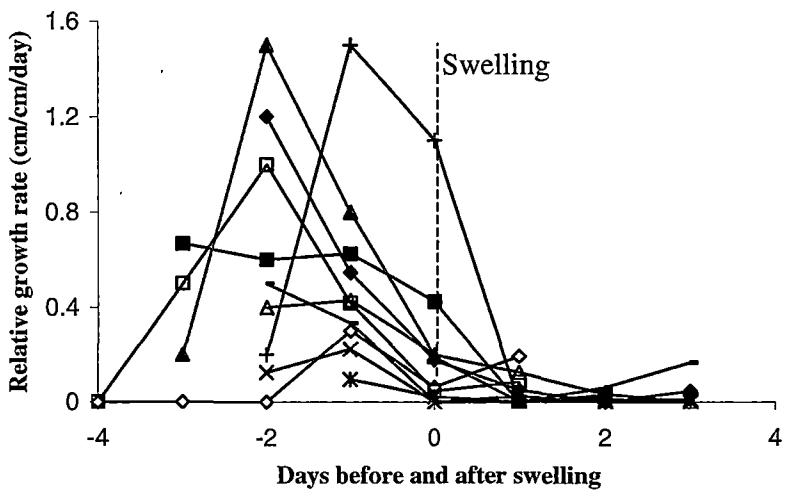


**Figure 5.** The relative growth rate of 10 individual stolons during tuberization in the May-June crop, where day 0 was the day of swelling.

This pattern was repeated in individual stolons examined in crops grown in September-October, June-July, and May-June, but the timing of the peak in relative growth rate appeared to vary with season (Fig. 6). The trend apparent in the data was for relative growth rate to peak the earliest in the September-October crop and the latest in the May-June crop. After swelling, stolon relative growth rate declined to a very low level.

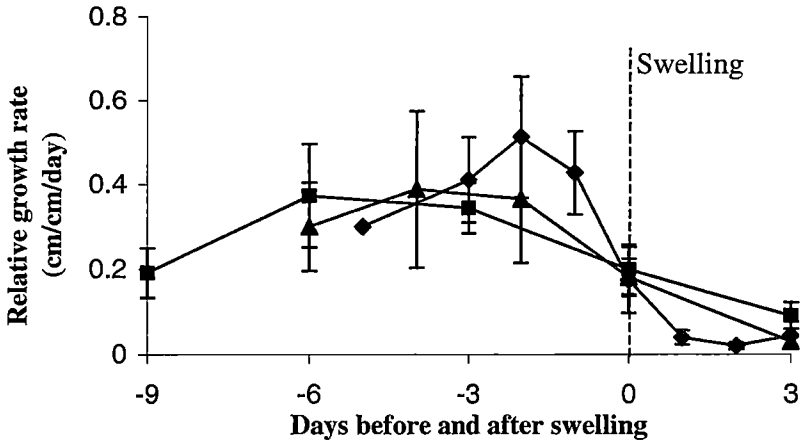


**Figure 6.** Stolon relative growth rates of the May-June (◆) (N = 10), July-August (▲) (N = 9) and September-October (■) (N = 9) plants, where day 0 was the day of swelling. Bars show SE.

# DISCUSSION

The earliest observation in this study of stolon initiation, 8 days after planting, was similar to that reported by Cao and Tibbitts (1996), 7 days after planting in hydroponics with cultivar Norland. The period of the stolon elongation, from initiation to growth cessation, varied with season. Stolon elongation lasted between 15 and 27 days, which is in the range of published data. The stolon growth phase has been documented to last between 20 to 25 days for cultivar Rural New Yorker No. 2 (Edmundson, 1938), and 10 to 15 days for cultivar Irish Cobber (Yamamoto and Noda, 1951).

Although considerable variation in individual stolon growth was reported (Chapter 3A and this chapter) even in the same plant, when total stolon length per plant was considered, a consistent pattern of growth was observed. The overall pattern of stolon growth was that of an initial phase of slow elongation followed by more rapid growth, which was consistent with previous observation (Lovell and Booth, 1969).

Stolon growth pattern varied with the season. Stolons from March-April, July-August and September-October crops showed a similar pattern of elongation, in that there was a short initial lag period followed by a more rapid rate of extension. Stolon growth rates reached their maximum between 20 and 22 days after initiation. However, for the December-January crop, the initial lag of stolon growth was much longer, and stolon growth rate reached the maximum value at 32 days after planting. Hence, the overall stolon elongation period from stolon initiation to the maximum growth rate was longer for the December-January crops at 20 days, compared to other crops at 10-11 days. The characteristic growth pattern with a rapid elongation phase lasting 10 days has been reported previously from cultivar Majestic grown in culture solution (Lovell and Booth, 1969). The rapid stolon growth phase was shorter in the present study, lasting 5, 6, 7 and 8 days in the July-August, September-October, March-April and December-January plants respectively.

The timing of tuber initiation also varied with the season. The main difference was that plants in summer (December-January) tuberized much later, about 36 days after planting, compared to other seasons where tuberization commenced 21-22 days after planting. The time of tuber onset in solution culture recorded by Cao and Tibbitts (1996) and Lovell and Booth (1969) was 21 and 35 days after planting respectively. Others reported that tubers appeared 52 days (Wan *et al.*, 1994) and 30 days (Struik *et al.*, 1988b) after planting in hydroponic systems. The delay of tuberization in summer was in agreement with previous studies that tuber initiation began earlier in the winter than in the summer (Nelson and Hwang, 1975; Manrique *et al.*, 1984). The consensus in the literature was that initiation occurred earlier in SD than in LD (Ewing, 1990; Ewing and Struik, 1992) and it is probable that differences in photoperiod were responsible for the seasonal differences recorded in this project. The photoperiod reached a maximum of 15 hours during summer (November-January), while the photoperiod during winter was as low as 9 hours. Since temperature was controlled within the glasshouse and only varied slightly between seasons, from 13°C in winter to 18.4 °C in summer, it is unlikely that it was a major factor causing delayed tuberization in summer crops.

Regardless of photoperiod or other environmental factors, the onset of stolon swelling and tuber initiation was always preceded by a peak in stolon elongation rate. Swelling occurred 1 to 3 days after stolons reached the maximum growth rate in crops grown in all seasons except summer, where swelling occurred 6 days after stolons reached the maximum growth rate. This suggests that either a peak in elongation rate was required to initiate swelling or that changes associated with tuber initiation occurred 1 to 6 days prior to visible swelling resulted in cessation of stolon elongation. After swelling, stolon elongation decreased to a very low rate, as has been described previously (Kefi *et al.*, 2000).

# CHAPTER 4

# NUTRIENT UPTAKE AND TUBERIZATION

## OUTLINE

***Nutrient uptake in intact plants can be readily determined in hydroponic systems by measuring changes in the nutrient concentration and volume of the nutrient solution over time. The rates of nutrient and water uptake were determined in the NFT hydroponic system used in this project at each of the key developmental stages defined in the previous chapter (Chapter 3A). Uptake rates were determined for $NO_3^-$, $PO_4^{3-}$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ under each set of growth environment conditions used in the project. Data were analyzed to identify relationships between timing of key stages of tuberization and nutrient uptake, plant size and nutrient uptake, and water uptake and nutrient uptake rate. The study was undertaken to establish if changes in rate of uptake of specific nutrients could be linked to key events in tuber formation and development.***

## INTRODUCTION

Sixteen inorganic mineral elements are essential for plant growth (Jones, 1998). These inorganic nutrients have structural and functional roles in all plants. The nutrient elements are normally divided into two groups, macronutrients and micronutrients, according to the quantity required. Macronutrients include C, H, O, N, P, K, Ca, Mg and S, while Fe, B, Mn, Cu, Zn, Mo and Cl are micronutrients.

Every element plays a particular role in plant growth. Some elements provide elemental constituents for macromolecules that function as enzymes or parts of cell structures, some act as cofactors for enzyme activity and thus play a regulatory role (Kramer and Boyer, 1995), while other elements regulate plant water status (Gutnecht, 1968; McNeil, 1976).

The physiological status of plants varies with developmental stage, so plants often require specific nutrients in different quantities at different stages. This leads to observed variations in critical nutrient concentrations in plant tissue with the developmental stage in potatoes (e.g. Karadogan *et al.*, 1999; Walworth and Muniz, 1993) and other crops (e.g. Gupta and Saxena, 1976; Dow, 1980; Marsh and Peterson, 1990).

Nutrient availability to plants can be rapidly changed in hydroponics, allowing nutrient supply to be matched to plant requirements. Specific nutrient requirements for potatoes during stolon development and tuber initiation have not been published. Obtaining this knowledge and the potential to apply it in hydroponic minituber productive system was a key object of the industry partner in this project.

# LITERATURE REVIEW

The effect of nutrient applications on potato crops has been widely studied in field-grown potatoes. Most of the published studies have concentrated on tuber yield in response to nutrient application and tissue nutrient concentration, with less attention paid to plant nutrient uptake during key stages of development. O'Brien *et al.* (1998) concluded that there were few published reports on the effects of nutrients other than N on tuber initiation. However, there have been numerous studies published on potato tissue nutrient contents (see review by Walworth and Muniz, 1993) which provide clues to the specific nutrient requirements of potato plants at different developmental stages.

While few studies have examined nutrient uptake at key stages in potato crop development, there is evidence that the uptake of various elements and their distribution and redistribution within the plant varies during crop growth. For example, shortly after germination in soil the concentration of Al and Fe in the plant was very high, even to a level considered toxic for the more mature plants, but the young plants seemed unaffected (Jones, 1998). Within a few weeks of germination, the concentration of Al and Fe in the plant declined sharply (Jones, 1998). At the early growth, plants preferred the ammonium rather than the nitrate form of nitrogen. This preference declines as the plant approaches maturity, when nitrate accumulates in the plant to fairly high concentrations (Jones, 1998).

## Nutrient Elements in Potato Tissue

In general, nutrient levels in potato tubers decrease from the early to late developmental stages. Tuber N, K (Hawkins, 1946; Jackson and Haddock, 1959), P (Walworth and Muniz, 1993) and Ca (Hawkins, 1946; Walworth and Muniz, 1993) were found to decrease through the growing season. More recently,

Karadogan *et al.* (1999) studied the nutrient levels in potato tubers from the beginning of tuber set (50 days after planting) until harvest (130 days) at intervals of 10 days. The tuber concentration expressed on a dry weight basis of N, P, Ca, Mg and K declined from tuber set to maturity. The decrease in tuber nutrient concentration may be due to the rapid accumulation of starch during tuber bulking (Cao and Tibbitts, 1997).

Changes in nutrient levels in potato shoots during crop development appear to be more variable than those in the tubers, with some nutrient concentrations decreasing and others increasing. Leaf N (Lorenz and Tyler, 1983; Kunkel and Thorton, 1986), K (Lorenz and Tyler, 1983) and P (Moorby, 1968) tended to decrease over the growing season. In contrast, there was a tendency for Ca levels to increase with age, both in the petioles and in the whole aboveground portions of the plants (Walworth and Muniz, 1993). This was supported by Manrique *et al.* (1984) who found that the concentration of N, P and K in shoots decreased as a function of time, and there was a progressive increase in concentrations of Ca, Mn, Fe and Si in the shoots as the plants reached maturity.

The different trends of nutrient concentrations in shoots may reflect differences in phloem mobility. N, P and K have high phloem mobility (Jones, 1998) and can be transported from the shoot to tuber continuously, hence shoot content of these elements decreases as tubers become the dominant sink tissue during crop development. Ca, Fe, Mn and Si have low phloem mobility (Ziegler, 1975; Jones, 1998). As xylem flow to stolons (Nelson *et al.*, 1990) and tubers (Kratzke and Palta, 1985) has been reported to be negligible, very little Ca and Si can be transported to the tubers from the shoots. This may lead to accumulation of these elements in the shoot system.

## Nutrient and Tuberization

The effect of nutrient application on potato growth and crop yield has been extensively studied in field experiments. Studies have mostly concentrated on the effects of the macronutrients N, P, K, Ca and Mg on tuber yield. In only a few cases, the effect of nutrient elements on potato tuberization has been reported.

In solution culture experiments, high rates of N application have been shown to delay or even inhibit tuberization in inductive conditions while low N promoted tuberization in inductive conditions (Krauss and Marschner, 1976; Sattlemacher and Marschner, 1979). Repeated cycles of high nitrogen/ low nitrogen can result in the formation of chain tubers (Krauss and Marschner, 1976), indicating phases of tuber induction and stolon growth coinciding with cycles of low and high nitrogen availability. However, reduced nitrogen application in non-inductive long day (18 hours) or high temperature (constant 30 $^{o}$C) conditions did not result in tuberization (Krauss and Marschner, 1982). Three hypothesises have been put forward to explain the effects of nitrogen on tuberization: (1) nitrogen withdrawal affects phytohormone levels, causing a reduction in GA levels and an increase in ABA levels (Krauss, 1985); (2) Nitrogen promotes shoot and root growth at the expense of stolon growth, resulting in reduced availability of carbohydrates for tuber formation (Jackson, 1999); (3) Rate of nitrogen uptake influences absorption of other nutrients (Kirkby and Mengel, 1967; Baker and Maynard, 1972), particularly P, Ca, and Mg, and high rate of N uptake may restrict the availability of another nutrient required for tuberization. Support for this theory comes from the observation that high nitrogen supply by foliar application did not prevent tuberization (Sattelmacher and Marschner, 1979).

There was little evidence to suggest that the actual time of tuber initiation in the field is affected by nitrogen level and the effect of nitrogen on tuber number may vary between seasons (Harris, 1992). Plants grown in May did not show any

difference in tuber number when N was applied during tuber initiation but in June tuber number increased with increased N application (Harris, 1992).

Increased phosphorus application to potato plants has been shown to increase tuber number (Hanley *et al.*, 1965; Berryman *et al.*, 1973; Perrenoud, 1983). Phosphorus plays an important role in the transport of materials in the phloem, particularly with respect to transport into storage tissue (Mengel and Haeder, 1977). An adequate supply of phosphorus is therefore likely to be required during tuberization to enable translocation of carbohydrate and other solutes from shoots to stolon tips. Hence P may have a promotive effect for tuberization.

Potassium application has been shown to influence total tuber yield in field-grown potatoes (Maier *et al.*, 1994; Panique *et al.*, 1997; Tawfik, 2001). The major promotive effect of potassium on yield has been shown to be through increased tuber size rather than tuber number in both *in vitro* study (Naik and Sarkar, 1998) and in field experiments (Panique *et al.*, 1997; Tawfik, 2001).

Potatoes require an adequate supply of calcium to tuberize. Restricting calcium uptake and transport to stolons and tubers in solution culture and field-grown plants reduced the rate of tuber initiation, and tuber development was retarded (Krauss and Marschner, 1971, 1973; Marschner, 1974; Paiva *et al.*, 1997). The addition of high concentrations of calcium to media in *in vitro* studies of tuberization resulted in a significant increase in the number of tubers formed (Balamani *et al.*, 1986). Increasing the concentration of Ca in nutrient solution (up to 36 mg/L) led to an increase in the number of small tubers formed (Paiva *et al.*, 1997).

Magnesium deficiency caused a considerable decrease in tuber yield but had no effect on the starch content of tubers (Talburt and Smith, 1959). Increasing Mg alone in an *in vitro* study did not promote tuberization, but Mg together with Ca promoted tuberization (Balamani *et al.*, 1986). These authors suggested that

magnesium might not play a promotive role in tuberization, but may activate several Ca-regulated enzymes, such as protein kinases, that promoted tuberization.

In comparison to the nutrient elements described above, others have received less study. Early studies indicated that neither the micronutrients (iron, manganese, copper, zinc, boron and molybdenum) nor sulfur increased or decreased tuber set or tuber yields (Barnes, 1944; Cotrufo *et al.*, 1963).

While not considered as an inorganic nutrient, the availability of hydrogen ions has been shown to affect tuberization. A soil pH of 4.7 to 5.5 was considered optimum for tuber development. Although raising the soil pH to a range of 5.8 to 6.5 further increased the yield of potatoes, it could not be recommended for commercial practice because the incidence of scab increased (Barnes, 1944). However, in solution culture experiments, exposing potato plants to low pH (3.4 and 4.0) for a short period promoted tuberization and increased tuber numbers significantly compared to the control plants where pH was maintained at 5.8 (Wan *et al.*, 1994).

Hydrogen ions affect many physiological processes in the plant and thus a pH change may influence tuberization. For example, hydrogen ions influence enzymatic activities. Avigad and Milner (1966) investigated the properties of sucrose synthase from sugar beet roots and found that sucrose cleavage was five times greater than sucrose synthesis when the pH was decreased from 7.2 to 6.0. Sucrose synthase from developing potato tubers has different pH optima for sucrose cleavage and synthesis (Pressey, 1969). As changes in sucrose metabolism are associated with tuber initiation, altered sucrose synthase activity due to changes in pH may be linked to tuberization.

## Water Uptake and Tuberization

Water stress is detrimental to potato tuber production. Potato crops are highly sensitive to water stress (van Loon, 1981). Even a slight water stress led to stomatal closure (Campbell *et al.*, 1976) and a significant decline in photosynthesis (Vos and Oyrzun, 1987). The decline in photosynthesis under water stress resulted from reducing $CO_2$ uptake following stomatal closure (Chapman and Loomis, 1953), and led to lower tuber yield and quality (van Loon, 1981; Miller and Martin, 1987; Gregory and Simmonds, 1992).

Water stress may affect tuberization to different extents at different developmental stages. Water stress during stolon development and tuberization reduced the number of tubers (Steckel and Gray, 1979) and both fresh and dry matter yield. Water stress during the mid-bulking period severely reduced both fresh and dry matter yield (Stark and McCann, 1992). However, there was evidence that the damaging effect of drought during tuber initiation and tuber bulking was almost the same (Miller and Martin, 1987).

In spite of the importance of water in potato crops, detailed information of water uptake on potato tuber initiation and tuber development is not available. While not directly measuring water uptake, Hamilton (1968) measured moisture extraction by potato plants from soil and reported that the largest amount of moisture extracted from the soil occurred at the bloom and tuber setting stage.

Transpirational water loss accounts for around 99.8 % of water uptake by plants (Miller, 1938), therefore the measurement of plant transpiration can be used as an indicator of plant water uptake. Nelson and Hwang (1975) measured transpiration rate during a 24-hour period weekly from emergence to tuber initiation and found that the transpiration rate calculated per unit of either fresh or dry weight of the

leaves or the total plant, peaked at 42 days after emergence when tuber initiation commenced and then slowly declined throughout the rest of the experiment.

The changes in water and nutrient uptake during crop development, while influenced by the physiological state of the plant, vary with environmental conditions (Sawas and Lenz, 1995). It is therefore necessary to characterize the changes occurring in new production systems, such as hydroponics, before attempting to manipulate the system. The aim of this section of experimental work was to identify any characteristic changes in nutrient or water uptake during the stages of development around tuber initiation.

# METHODS AND MATERIALS

The rates of uptake of $NO_3^-$, $PO_4^{3-}$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ were measured at development stage 2 to 5 in a series of crops grown in different seasons. Three trays of each crop grown in July-August (2001), November-December (2001), March-April (2002), May-June (2002) and September-October (2002) were used. Nutrient uptake data was not collected at stage 3 in the July-August crop, stage 2 in the March-April crop and stage 5 in the November-December crop. In addition, the plants in the remaining three trays in the November-December crop were treated with the chemical growth retardant Cultar® (ICI Australian Operations Pty Ltd), active ingredient paclobutrazol. The treatment promoted tuberization and enabled comparison of nutrient uptake rate at the same time of year in plants initiating tubers at different rates. Paclobutrazol was applied as a foliar spray 32 days after the crop was planted. Plants were in the stolon elongation phase (development stage 2) at the time of application. Two litres of 500 μg/L paclobutrazol solution containing 1.2 ml/L cittowett (wetting agent) was applied to each treatment tray, and the same volume of distilled water containing 1.2 ml/L cittowett was applied to control plants. Uptake rate was first measured 3 days after paclobutrazol application. Since plant growth rate varied between the different seasons, the age of crops (from planting) at each stage of development differed. The age and stage of development at the time of recording nutrient uptake is given in Table 10.

Nutrient uptake was determined by measurement of change in nutrient solution over a 24 hours uptake period. Prior to the start of each measurement, nutrient solution of standard composition was prepared. The hydroponic trays were drained for 30 minutes prior to addition of new solution so that very little solution remained in the system. The new nutrient solution was weighed in the hydroponic reservoir and the pump turned on. After the addition of new solution, the system was equilibrated for 30 minutes and the initial nutrient solution sample was collected. After twenty-four

**Table 10.** Crop age (days after planting) and each stage of development when nutrient uptake rate was recorded for each of the 6 crops assessed. NM = not measured.

| | **Crop age (days after planting)** | | | |
|---|---|---|---|---|
| **Crop** | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
| **March-April** | 20 | 24 | 29 | 34 |
| **May-June** | NM | 22 | 28 | 35 |
| **July-August** | 23 | NM | 29 | 33 |
| **September-October** | 22 | 28 | 33 | 40 |
| **November-December** | 30 | 46 | 52 | NM |
| **November-December (PBZ)** | 30 | 39 | 44 | 56 |

hours, the system pump was switched off and the tray was drained for 30 minutes. The solution was weighed in the hydroponic reservoir and the second sample was taken. Every measurement was started between 15:00 and 17:00. Three hydroponic trays were used in each trial, allowing samples to be taken in triplicate.

Ca, Mg, K, P and S were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Thermo Jarrell Ash, Franklin, Ma, USA) as described in Chapter 2. $NO_3^-$ was measured by UV spectrophotometer (Cawse, 1967) (see Chapter 2). Assuming the density of the solution was equivalent to that of water, the amount of each element in solution was calculated as: concentration (mg/l) x volume (l) = content (mg). The difference in the amount of elements was taken as being absorbed by plants.

Plant fresh weight was determined each time when nutrient uptake rate was measured. The total weight of each tray and the plants contained was measured after the trays had been drained and prior to the addition of fresh nutrient solution. The weight of each tray was also measured after nutrient solution had been

circulated and drained prior to planting of the crop. Plant fresh weight was calculated by subtracting the weight of the tray measured before planting from the total weight of the tray and plants.

As plant water uptake depends on the environment, particularly, irradiance (Sawas and Lenz, 1995), and nutrient uptake rate may be affected by water uptake rate, rainy and cloudy days were avoided when taking nutrient uptake measurements to minimize variability due to differences in irradiance. Variation in nutrient and water uptake rate due to the environment was assessed in the March-April crop. Uptake rates were measured in the same tray over nine consecutive days from stage 3 (swelling stage) to stage 5 (bulking stage). Photosynthetically active radiation and temperature were recorded during the uptake measurements. Photosynthetically active radiation was recorded every 5 minutes using a data logger (Li Cor 1400, USA) connected to a line quantum sensor (Li Cor 191SA, USA) set next to plants in the glasshouse. Temperature was recorded every 15 minutes using a Tinytag (Ultra) data logger (Gemini Data Logger UK Ltd) set next to plants in the greenhouse. Daily means of photosynthetically active radiation and temperature were calculated between 6:00 and 18:00.

Uptake rate at different stages of the same trial were tested for significance and correlation of nutrient uptake rate and plant size, the amount of water uptake and nutrient uptake, and photosynthetically active radiation /temperature and uptake were analysed.

# RESULTS

## Uptake Rate at Different Developmental Stages

Uptake rate generally showed a similar pattern of change with plant development stage for all nutrients and across all seasons (Fig. 7A and 7B). Nutrient uptake declined with plant development from stolon growth to tuber bulking.

Nitrate ($NO_3^-$) uptake rate tended to decrease from early stage to late stage in all trials except in the May-June grown plants. The decrease was significant ($p < 0.05$) in the July-August, September-October and March-April crops. Uptake rate decreased from 1.07 ± 0.25 mg/gFW/day at stage 2 to 0.40 ± 0.03 mg/gFW/day at stage 5 in the July-August plants, from 1.45 ± 0.19 mg/gFW/day at stage 2 to 0.50 ± 0.02 mg/gFW/day at stages 5 in the September-October trial, and from 1.84 ± 0.27 mg/gFW/day at stage 2 to 0.13 ± 0.02 mg/gFW/day at stages 5 in the March-April trial. In the November-December trial, uptake rate decreased significantly from 0.54 mg/gFW/day at stage 2 to 0.39 mg/gFW/day at stage 4. Uptake rate of nitrate in the May-June grown plants was not significantly different at stages 3, 4 and 5 (Fig. 7A $NO_3^-$).

Phosphorus ($PO_4^{3-}$) uptake rate decreased significantly ($p < 0.05$) during potato tuberization in the July-August, May-June and November-December crops. Uptake rate decreased from 1.08 ± 0.16 mg/gFW/day at stage 2 to 0.20 ± 0.01 mg/gFW/day at stage 5 in the July-August trial, from 0.59 ± 0.13 mg/gFW/day at stage 3 to 0.16 ± 0.04 mg/gFW/day at stage 5 in the May-June crop, and from 0.17 ± 0.005 mg/gFW/day at stage 2 to 0.07 ± 0.005 mg/gFW/day at stage 5 crop in the November-December crop. The uptake rates at stages 2 and 3 were lower in the September-October, November-December and March-April crops than the May-June and July-August crops (0.26 and 0.05 mg/gFW/day compared to 1.10 and 0.59 mg/gFW/day) and the trends in uptake rate with increasing stage of development were less pronounced. Uptake rate did not decrease significantly

from early to late stages in the September-October and March-April crops. (Fig. 7A $PO_4^{3-}$).

Potassium uptake rate decreased significantly ($p < 0.05$) during tuberization in the July-August, March-April and November-December trials. Uptake rates decreased from 3.14 ± 0.22 mg/gFW/day at stage 2 to 0.55 ± 0.05 mg/gFW/day at stage 5 in July-August, from 2.28 ± 0.10 mg/gFW/day at stage 2 to 0.22 ± 0.03 mg/gFW/day at stage 5 in March-April, and from 0.64 ± 0.01 mg/gFW/day at stage 3 to 0.41 ± 0.01 mg/gFW/day at stage 5 in November-December. In September-October and May-June, K uptake rate did not decrease significantly from early to late stages. The trends of uptake rate with increasing stage were less in the May-June and November-December trials compared to other crops. The highest uptake rate was recorded in the July-August trial (Fig. 7A $K^+$).

The uptake rates of Ca in the July-August, March-April and November-December crops decreased significantly ($p < 0.05$) during tuberization, from 3.30 ± 0.53 mg/gFW/day at stage 2 to 0.62 ± 0.04 mg/gFW/day at stage 5 in July-August, from 0.45 ± 0.11 mg/gFW/day at stage 2 to 0.05 ± 0.02 mg/gFW/day at stage 5 in March-April and from 0.45 ± 0.01 mg/gFW/day at stage 2 to 0.24 ± 0.02 mg/gFW/day at stage 4 in November-December. The trends of Ca uptake rate decrease with increasing stage were less in the November-December and March-April crops in comparison to the July-August and May-June trials. However, the uptake rates in the September-October and May-June crops did not decrease significantly from early to late stages. Uptake rates at each stage were higher in the July-August and May-June trials in comparison to the September-October, November-December and March-April trials (Fig. 7A $Ca^{2+}$).

Mg uptake rates decreased significantly ($p < 0.05$) during tuberization in the September-October, November-December and March-April crops, from 0.17 ± 0.03 mg/gFW/day at stage 2 to 0.07 ± 0.003 mg/gFW/day at stage 5, from 0.08 ± 0.003 mg/gFW/day at stage 2 to 0.05 ± 0.007 mg/gFW/day at stage 4, and from

0.29 ± 0.04 mg/gFW/day at stage 2 to 0.02 ± 0.01 mg/gFW/day at stage 5 respectively. Uptake rate decreased marginally in the July-August ($p = 0.07$) and May-June ($p = 0.08$) trials, from 1.94 ± 0.68 mg/gFW/day at stage 2 to 0.26 ± 0.09 mg/gFW/day at stage 5 and from 0.67 ± 0.18 mg/gFW/day at stage 3 to 0.21 ± 0.07 mg/gFW/day for stage 5 respectively. Mg uptake rates at each stage were higher in the July-August and May-June crops in comparison to the November-December, March-April and September-October trials (Fig. 7B $Mg^{2+}$).

Sulfur ($SO_4^{2-}$) decrease significantly ($p < 0.05$) during tuberization in the July-August trial, from 0.89 ± 0.09 mg/gFW/day at stage 2 to 0.23 ± 0.01 mg/gFW/day at stage 5. Uptake rate in the November-December, March-April and May-June displayed a similar pattern, but no statistically significant changes were detected. Uptake rate recorded in September-October displayed a different trend compared to the other trials conducted, with uptake rate decreasing from stage 2 to stage 4, but increasing from stage 4 to stage 5. Uptake rates in the July-August crop were all higher at each stage in comparison to other trials (Fig. 7B $SO_4^{2-}$).

Changes in concentration of hydrogen ions in nutrient solution were measured. Although it has been shown that a number of factors may affect concentration of hydrogen ions in nutrient solution, for example, the form of nitrogen used in nutrient solution (Baker and Mills, 1980; Pilbeam and Kirkby, 1992), other than uptake by the plants, these are considered to be minor. The change in concentration of hydrogen ions was assumed to be due to plant uptake or excrete in this study. Plants took up hydrogen ions during tuberization in the July-August, September-October and November-December trials, but not in the March-April and May-June trials. Hydrogen uptake rate decreased significantly ($p < 0.05$) during tuberization in September-October, from 1.79E-07 ± 0.40E-07 at stage 2 to 0.02E-07 ± 0.007E-07 mg/gFW day at stage 5. Uptake rate in the November-December and September-October crops did not change significantly at all stages recorded. Plants showed $H^+$ efflux when grown in March-April and May-June (Fig. 7B $H^+$).

Water uptake rates decreased significantly ($p < 0.05$) with increasing developmental stages during tuberization in all trials but the May-June trial (Fig. 7B $H_2O$). Uptake rate decreased from 7.48 ± 0.66 g/gFW/day at stage 2 to 2.27 ± 0.07 g/gFW/day at stage 5 in July-August, from 9.02 ± 0.72 g/gFW/day at stage 2 to 2.58 ± 0.09 g/gFW/day at stage 5 in September-October, from 9.19 ± 0.73 g/gFW/day at stage 2 to 0.88 ± 0.05 g/gFW/day at stage 5 in March-April, and from 2.73 ± 0.08 g/gFW/day at stage 2 to 0.99 ± 0.02 g/gFW/day at stage 4 in November-December. Uptake rate in the May-June crop did not vary significantly from early to late stage. Uptake rate at stage 2 was very low, 2.73 ± 0.08 g/gFW/day, in November-December in comparison to other trials, between 7.48 ± 0.66 and 9.19 ± 0.73 g/gFW/day. Uptake rates of stage 3 and 4 in the November-December and March-April trials were extremely low compared to other trials.

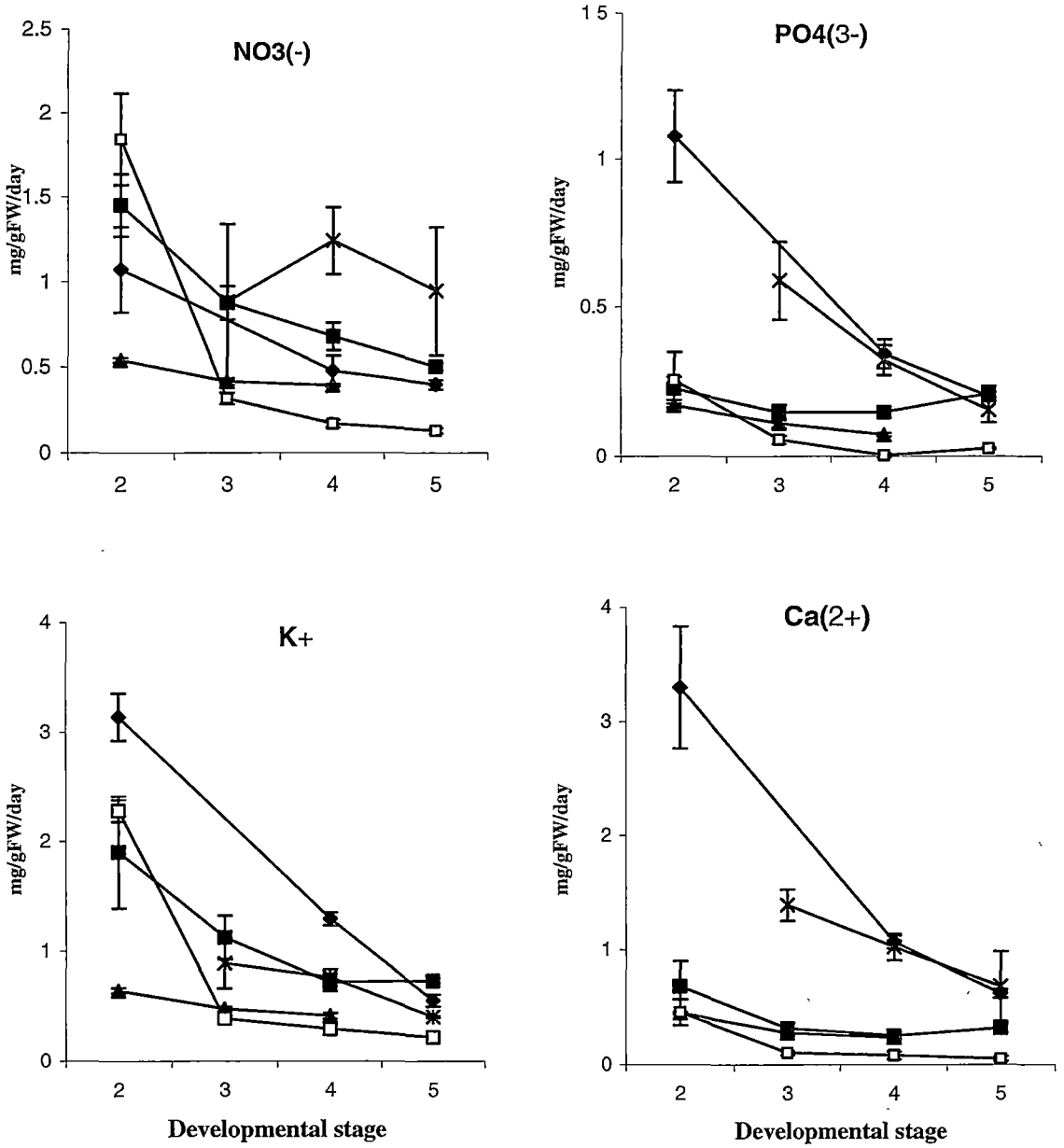


**Figure 7A.** Uptake rate of $NO_3^-$, $PO_4^{3-}$, $K^+$ and $Ca^{2+}$ at each stage of the July-August (◆), September-October (■), November-December (▲), March-April (□) and May-June (×) trials. N = 3. Bars show SE.

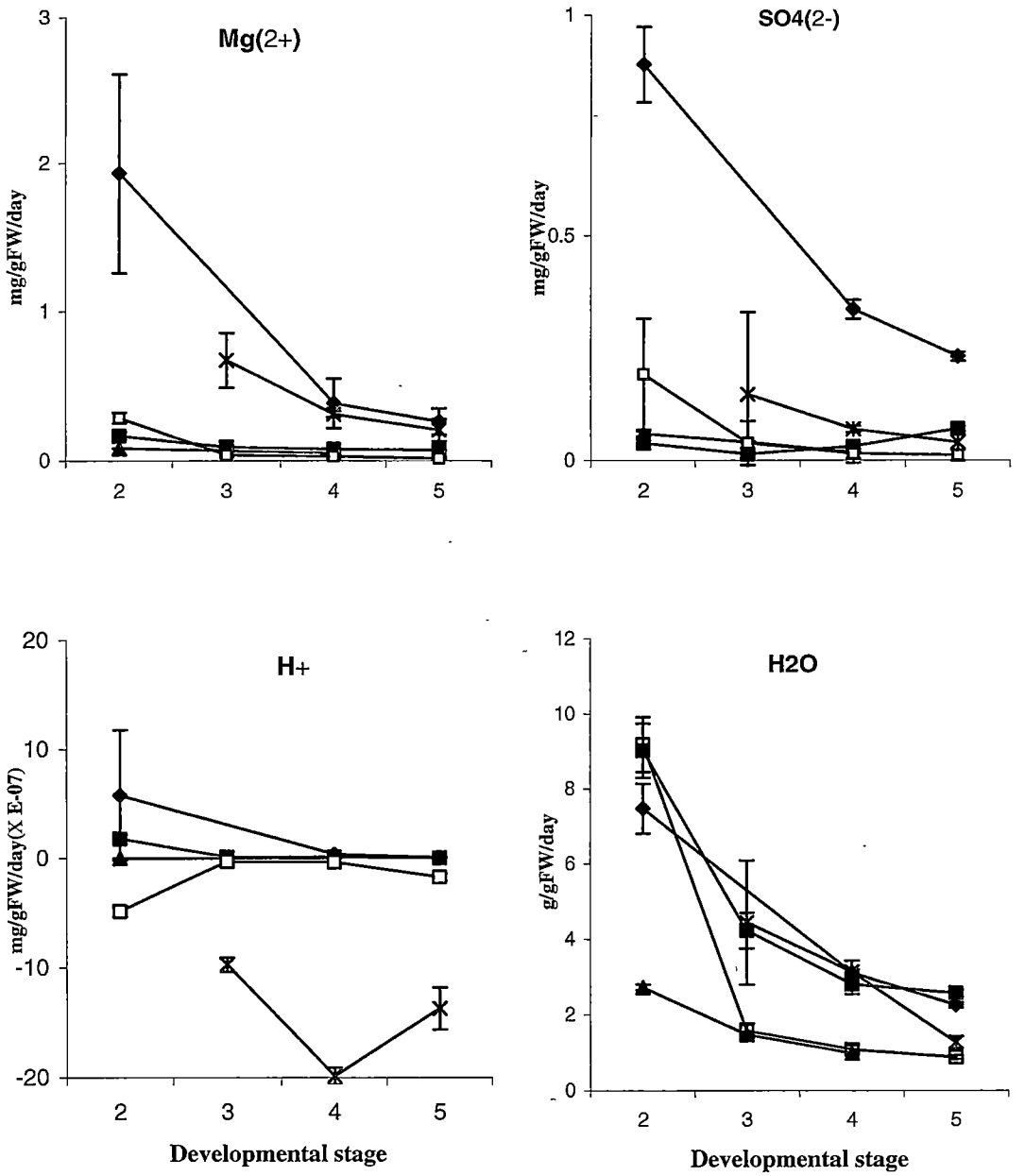


**Figure 7B.** Uptake rate of $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ at each stage of the July-August (◆), September-October (■), November-December (▲), March-April (□) and May-June (×) trials. N = 3. Bars show SE.

**Table 11.** Plant growth rate in crops used in the nutrient uptake trials. Plant weights are the mean of three replicates. NR = not recorded.

| Crop | Stage | Days after planting | Mean of plant fresh weight (g/plant) |
|---|---|---|---|
| **March-April** | 2 | 20 | 9.8 |
| | 3 | 24 | 99.2 |
| | 4 | 29 | 159.2 |
| | 5 | 34 | 187.0 |
| **May-June** | 2 | NR | NR |
| | 3 | 22 | 10.6 |
| | 4 | 28 | 11.5 |
| | 5 | 35 | 16.3 |
| **July-August** | 2 | 23 | 8.7 |
| | 3 | NR | NR |
| | 4 | 29 | 29.7 |
| | 5 | 33 | 51.7 |
| **September-October** | 2 | 22 | 45.7 |
| | 3 | 28 | 54.8 |
| | 4 | 33 | 92.3 |
| | 5 | 40 | 180.1 |
| **November-December** | 2 | 30 | 246.5 |
| | 3 | 46 | 391.1 |
| | 4 | 52 | 518.5 |
| | 5 | NR | NR |

## Uptake Rate and Plant Size

While significant differences in uptake rates at each development stage were recorded between crops, differences in rate of development and therefore plant weight at each stage of development may have contributed to the differences recorded (Table 11). In order to assess the relationship between plant size and nutrient uptake, the uptake rates of $NO_3^-$, $PO_4^{3-}$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ measured in the March-April, May-June, July-August, September-October and November-December trials were plotted against plant fresh weight (Fig. 8A, 8B). Generally, the uptake rates of water and all nutrient elements but $SO_4^{2-}$ and $H^+$ were inversely related to plant fresh weight. Small plants had high uptake rates while big plants had low uptake rates.

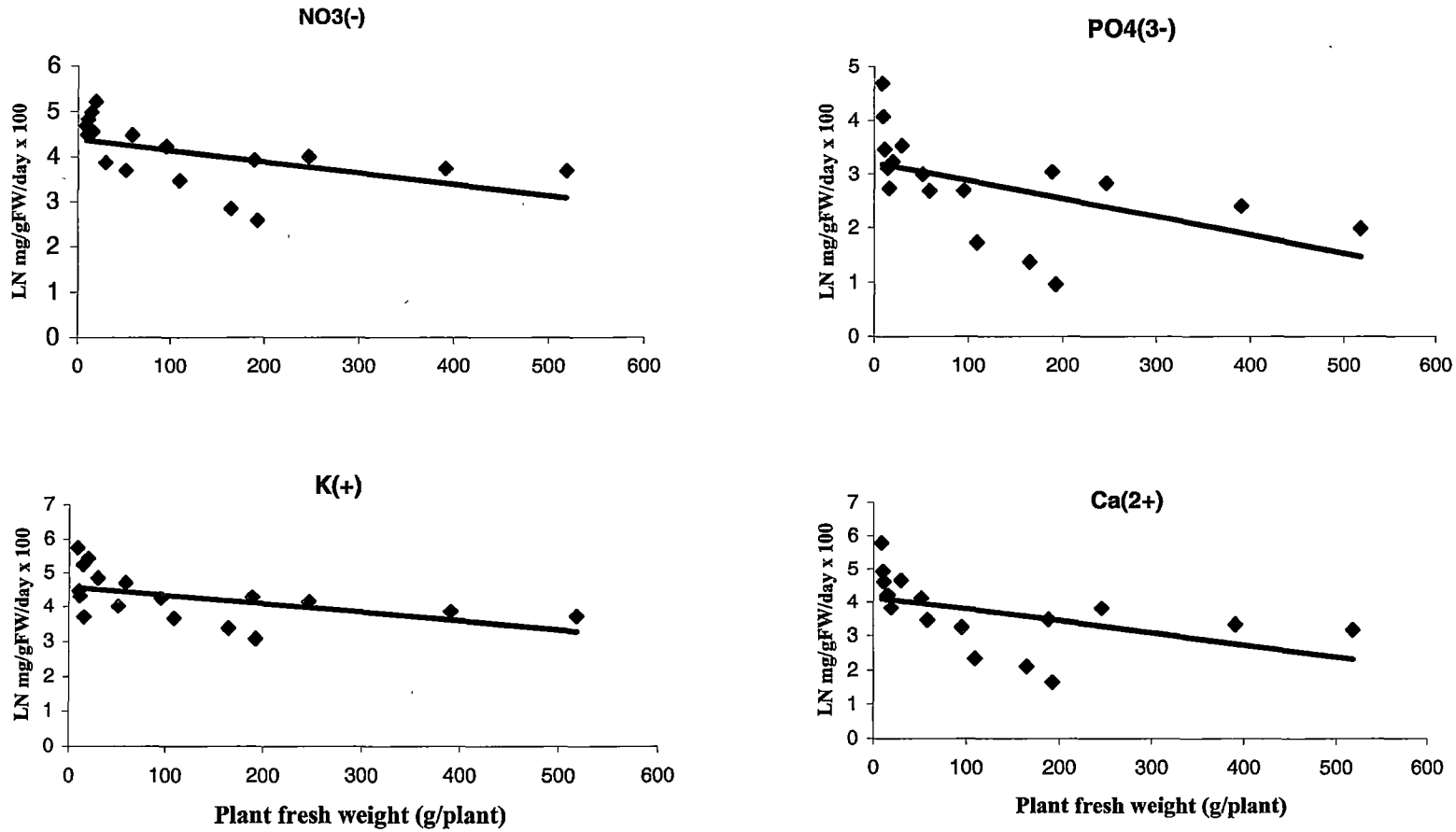


**Figure 8A.** The relationship between plant fresh weight and uptake rate of nutrient elements and water. P-value for $NO_3^-$, $PO_4^{3-}$, $K^+$ and $Ca^{2+}$ is 0.04, 0.03, 0.03 and 0.05, and R-square for $NO_3^-$, $PO_4^{3-}$, $K^+$ and $Ca^{2+}$ is 0.26, 0.28, 0.26 and 0.24 respectively. Each value is mean of three replicates. N = 17.

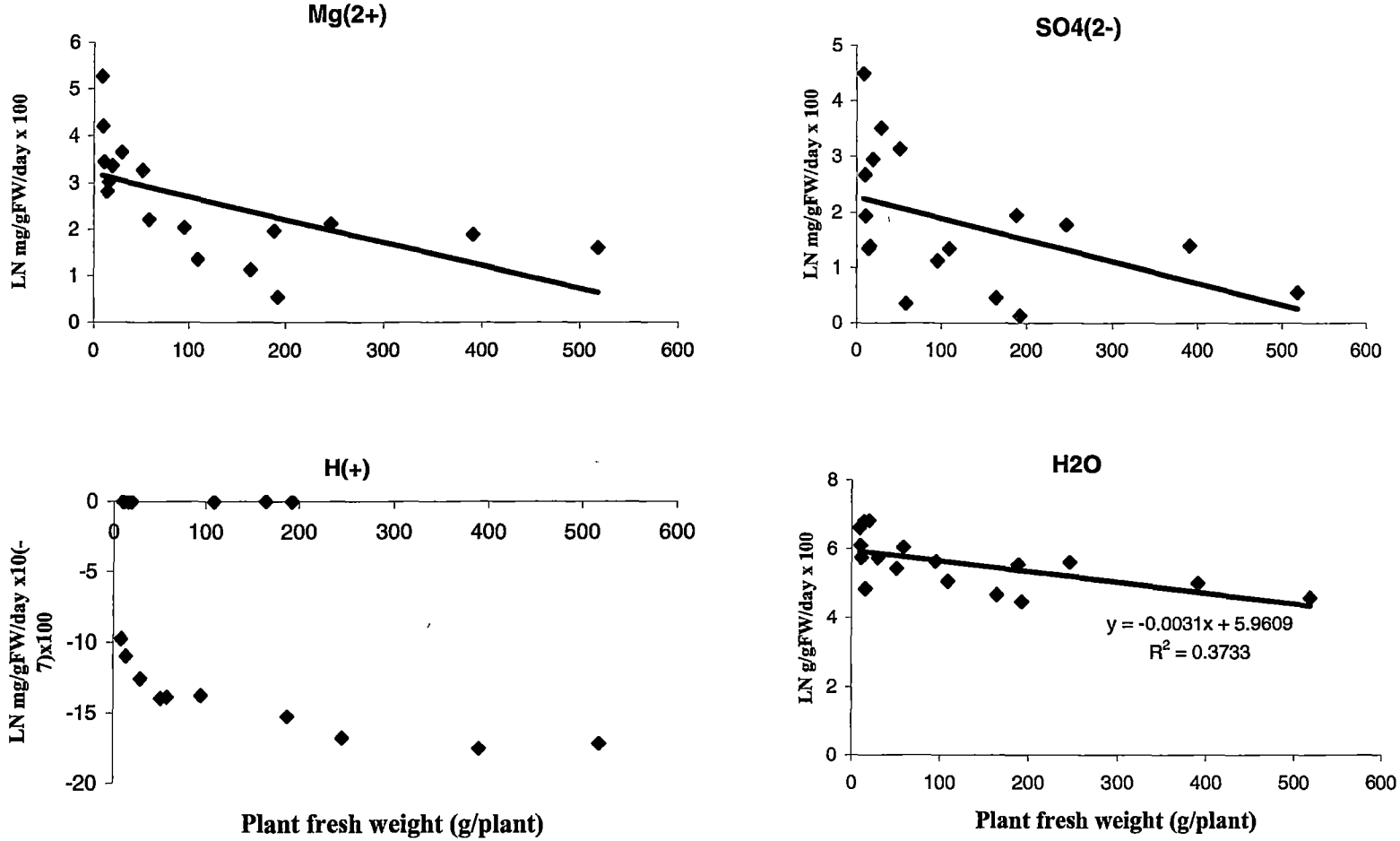


**Figure 8B.** The relationship between plant fresh weight and uptake rate of nutrients and water. P value for $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ is 0.01, 0.05, 0.001 and 0.01, and R-square for $Mg^{2+}$, $SO_4^{2-}$, $H^+$ and $H_2O$ is 0.36, 0.23, 0.24 and 0.37 respectively. Each value is the mean of three replicates. N = 17.

## Nutrient Uptake and Water Uptake

Significant relationships between uptake of water and uptake of each nutrient per tray were found (Table 12). The analysis utilized data collected from individual trays across the four developmental stages and five crops used in the study. While the overall relationships were significant, the clustered distribution of data due to large differences in plant fresh weights at the later stages of development in summer crops compared to other seasons strongly influenced the analysis. In order to remove the effect of the clustered distribution, whereby the analysis reflected higher water and nutreint uptake by large plants, the data for plant water uptake less than 300 g/tray/day was analysed separately (Table 13). The relationships between water uptake and $NO_3^-$, $PO_4^{3-}$, $K^+$and $H^+$ were significant while $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ uptake rate were not related to water uptake. Uptake of $NO_3^-$, $PO_4^{3-}$, $K^+$and $H^+$ increased with increasing water uptake (Fig. 9), and this relationship was evident across all seasons and developmental stages examined in this project. Uptake of $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ was not linked to water uptake, with most variation occurring between seasons and between stages.

**Table 12.** SAS analysis of plant water uptake correlated to nutrients uptake. N = 51.

| | $NO_3^-$ | $PO_4^{3-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $SO_4^{2-}$ | $H^+$ |
|---|---|---|---|---|---|---|---|
| **Pearson Correlation ($R^2$)** | 0.91 | 0.93 | 0.95 | 0.68 | 0.70 | 0.70 | 0.43 |
| **Significance (2-tailed) (P)** | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |

**Table 13.** SAS analysis of plant water uptake correlated to nutrients uptake. N = 39.

| | $NO_3^-$ | $PO_4^{3-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $SO_4^{2-}$ | $H^+$ |
|---|---|---|---|---|---|---|---|
| **Pearson Correlation ($R^2$)** | 0.88 | 0.45 | 0.92 | -0.10 | -0.04 | -0.04 | 0.62 |
| **Significance (2-tailed) (P)** | < 0.01 | < 0.01 | < 0.01 | = 0.54 | = 0.82 | = 0.82 | < 0.01 |

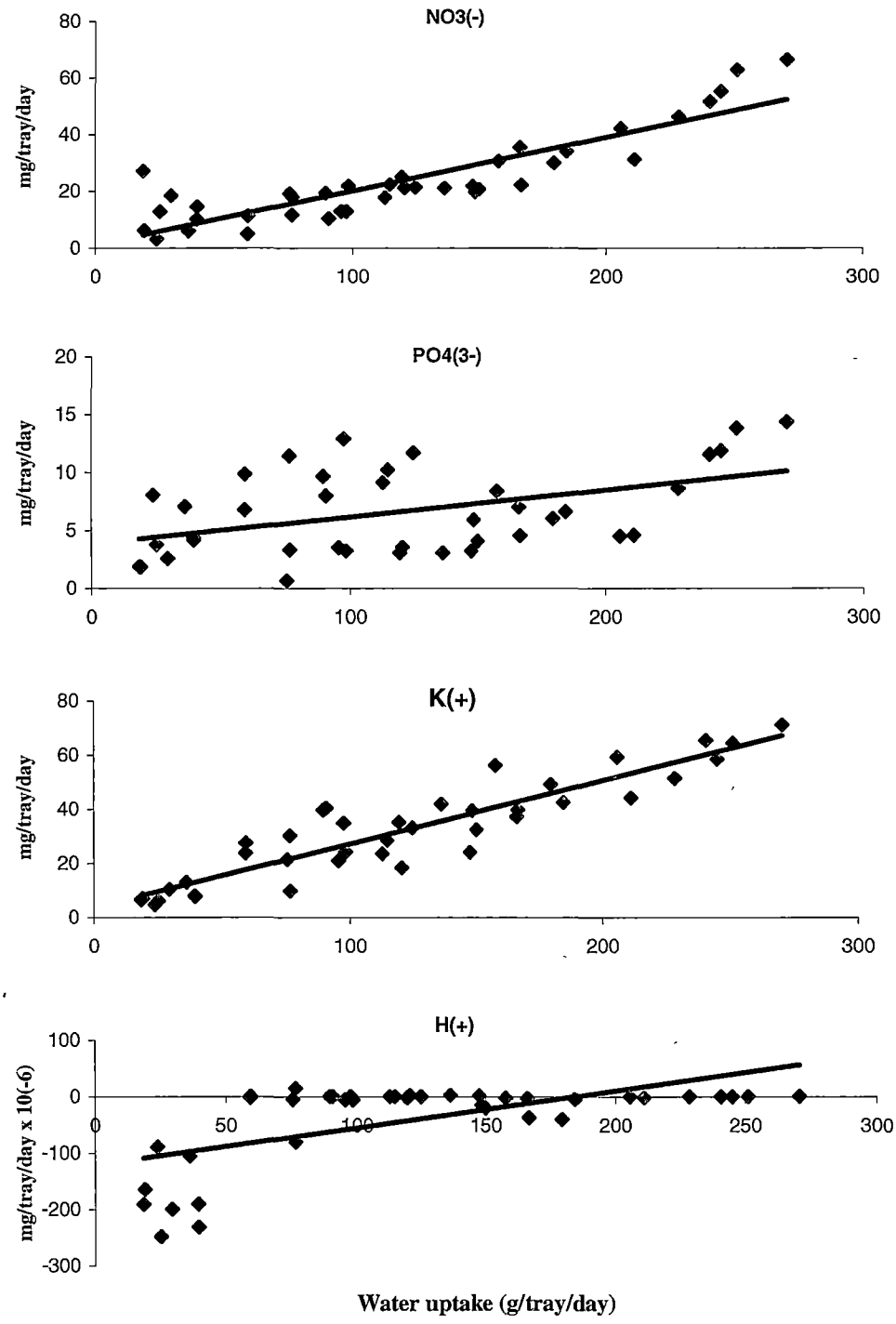


**Figure 9**. The relationship between water uptake and uptake of $NO_3^-$, $PO_4^{3-}$, $K^+$ and $H^+$. Each value was the mean of three replicates. There were significant ($p < 0.01$) correlation between water and each element uptake. R-square for $NO_3^-$, $PO_4^{3-}$, $K^+$ and $H^+$ was 0.78, 0.21, 0.84 and 0.39 respectively. N = 39.

# Nutrient Uptake in Paclobutrazol Treatment

Paclobutrazol (PBZ) was applied to plants at early stage 2 (stolon elongation). Uptake rates of $NO_3^-$, $K^+$ and $H_2O$ were significantly decreased by PBZ ($P < 0.05$), while $Ca^{2+}$, $Mg^{2+}$, $H^+$, $SO_4^{2-}$ and $PO_4^{3-}$ uptake were not significantly affected by PBZ at stage 2 and stage 4. However, at stage 3, uptake rates of all nutrient elements were not significantly different in control and PBZ-treated plants. (Table 14).

**Table 14.** Uptake rate (mean ± standard error) of elements and water affected by PBZ treatment in plants grown in November-December 2001. Where $H^+$ is expressed in unit of $10^{-10}$ mg/gFW/day, $H_2O$ in unit of g/gFW/day and other elements in unit of mg/gFW/day. Significance ($p < 0.05$) is shown in highlighted figures.

| | | Stage 2 | Stage 3 | Stage 4 |
|---|---|---|---|---|
| **$NO_3^-$** | Control | **0.54 ± 0.01** | 0.42 ± 0.02 | **0.39 ± 0.01** |
| | PBZ | **0.45 ± 0.01** | 0.39 ± 0.003 | **0.34 ± 0.01** |
| **$PO_4^{3-}$** | Control | 0.17 ± 0.006 | 0.11 ± 0.01 | 0.07 ± 0.005 |
| | PBZ | 0.20 ± 0.03 | 0.12 ± 0.01 | 0.08 ± 0.01 |
| **$K^+$** | Control | **0.64 ± 0.02** | 0.47 ± 0.02 | **0.41 ± 0.03** |
| | PBZ | **0.49 ± 0.01** | 0.45 ± 0.01 | **0.34 ± 0.01** |
| **$Ca^{2+}$** | Control | 0.45 ± 0.01 | 0.28 ± 0.03 | 0.24 ± 0.01 |
| | PBZ | 0.47 ± 0.04 | 0.29 ± 0.03 | 0.25 ± 0.01 |
| **$Mg^{2+}$** | Control | 0.08 ± 0.002 | 0.07 ± 0.005 | 0.05 ± 0.005 |
| | PBZ | 0.07 ± 0.005 | 0.06 ± 0.002 | 0.05 ± 0.005 |
| **$SO_4^{2-}$** | Control | 0.06 ± 0.01 | 0.04 ± 0.002 | 0.02 ± 0.003 |
| | PBZ | 0.05 ± 0.005 | 0.04 ± 0.004 | 0.02 ± 0.003 |
| **$H^+$** | Control | 5.15 ± 0.76 | 2.46 ± 0.44 | 3.54 ± 0.37 |
| | PBZ | 5.38 ± 0.29 | 2.47 ± 0.065 | 3.54 ± 2.13 |
| **$H_2O$** | Control | **2.73 ± 0.08** | 1.48± 0.07 | **0.99 ± 0.02** |
| | PBZ | **2.25 ± 0.10** | 1.39 ± 0.08 | **0.89 ± 0.04** |

## Environmental Factors and Uptake Rate

In order to investigate the effect of temperature and irradiance on uptake rate, the average daily photosynthetically active radiation and temperature from 6:00 to 18:00 were analysed with nutrient and water uptake rate. There were significant linear regressions between photosynthetically active radiation and uptake of $NO_3^-$, $K^+$ and $H_2O$. Uptake rate of $PO_4^{3-}$showed marginally linear regression with photosynthetically active radiation. Uptake of $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$ and $H^+$ did not correlate to photosynthetically active radiation (Table 15). Temperature did not show significant effect on uptake rate of nutrient elements. However, it is interesting to find a strong correlations between temperature and $H^+$ uptake rate (Table 15).

**Table 15.** Effect of temperature (°C) and photosynthetically active radiation (PAR) ($\mu mol.m^{-2}.s^{-1}$) on uptake rate of nutrient elements (mg/gFwt/day) and water (g/gFwt/day) in the March-April (2002) crop. The significant correlations were highlighted

| Day | Temperature | PAR | $NO_3^-$ | $PO_4^{3-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $SO_4^{2-}$ | $H_2O$ | $H^+$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 17.7 | 363.3 | 0.253 | 0.031 | 0.355 | 0.101 | 0.039 | 0.007 | 1.204 | -1.9E-08 |
| **2** | 17.2 | 447.4 | 0.357 | 0.071 | 0.376 | 0.076 | 0.036 | 0.064 | 1.674 | -2.8E-08 |
| **3** | 17.5 | 350.1 | 0.196 | 0.029 | 0.278 | 0.065 | 0.020 | 0.002 | 1.326 | -5.0E-09 |
| **4** | 17.3 | 437.8 | 0.343 | 0.067 | 0.429 | 0.136 | 0.042 | 0.044 | 1.859 | -4.1E-08 |
| **5** | 17.1 | 259.6 | 0.192 | 0.053 | 0.353 | 0.144 | 0.053 | 0.038 | 0.991 | -1.3E-08 |
| **6** | 15.9 | 212.5 | 0.111 | 0.022 | 0.174 | 0.051 | 0.006 | 0.010 | 0.802 | -1.1E-07 |
| **7** | 18.2 | 233.1 | 0.124 | 0.037 | 0.248 | 0.039 | 0.021 | 0.007 | 0.932 | -9.4E-08 |
| **8** | 14.6 | 315.3 | 0.118 | 0.024 | 0.213 | 0.017 | 0.013 | 0.010 | 0.891 | -2.0E-07 |
| **9** | 14.9 | 310.8 | 0.160 | 0.032 | 0.263 | 0.089 | 0.033 | 0.014 | 0.960 | -2.1E-07 |
| P value (temperature and uptake) | | | 0.23 | 0.27 | 0.15 | 0.38 | 0.33 | 0.67 | 0.24 | **0.004** |
| R square | | | 0.19 | 0.17 | 0.27 | 0.11 | 0.13 | 0.03 | 0.19 | 0.71 |
| P value (PAR and uptake) | | | **0.002** | 0.06 | **0.02** | 0.37 | 0.25 | 0.09 | **0.0006** | 0.38 |
| R square | | | 0.82 | 0.42 | 0.57 | 0.11 | 0.19 | 0.35 | 0.83 | 0.11 |

# DISCUSSION

The uptake rates of the nutrients measured in this study tended to decrease from early to late stages in all trials. Uptake rates did not decline with plant development in only a few cases, for example $NO_3^-$ uptake in May-June. A similar trend has been shown in potato plants in an earlier study. During the first 50 days after planting, plants absorbed 9 % of the total major nutrients while making only 3 % of the season's growth (Smith, 1968), indicating that potato plants might take up a relatively high rate of nutrients at an early developmental stage. This trend of nutrient uptake rate decrease from early to late stage is consistent with the conclusion of other studies that most nutrient elements including N, P, and K in potato leaves tended to decrease over the growing season (Lorenz and Tyler, 1983; Kunkel and Thorton, 1986).

Despite the finding that nutrient uptake rate generally decreased from early to late developmental stages, no significant relationship between nutrient uptake rate and stage of development was found. This suggests that nutrient uptake was not specific to stages of stolon/tuber development and that there was not any direct relationship between tuberization and plant nutrient uptake rate at the whole plant level. In this study plants were grown in nutrient solution with an adequate supply of nutrient elements so that they did not appear to preferentially increase uptake of any specific nutrient at times of peak requirement during tuberization.

Tubers are non-transpiring organ, therefore unlikely to receive nutrient via xylem flow, and the low rate of xylem flow into stolons (Nelson *et al.*, 1990) and tubers (Kratzke and Palta, 1985) has been demonstrated. The majority of the nutrient elements used for stolon and tuber growth must be transported from shoot via the phloem. Even the relatively phloem immobile Ca can be transported within phloem sap in sufficient amounts to account from 12 % to 100 % of the total Ca found in the tuber (Davies and Millard, 1985).

Nutrient elements can be accumulated and stored in the shoot system in the early developmental stage (Smith, 1968) and these elements can be translocated out from the

shoot system when they are required during tuberization. Nutrient translocation would lead to a gradual decrease in leaf nutrient content. The trend of declining nutrient levels in potato leaf was shown for N, P and K (Lorenz and Tyler, 1983; Manrique *et al.*, 1984; Kunkel and Thorton, 1986; Walworth and Muniz, 1993). The relocation of nutrients from shoot to stolon for tuber growth could also be seen in developing tubers in hydroponics in this study, as tubers were observed to grow without contact to the nutrient solution and therefore without being able to directly uptake nutrients.

It can be concluded that manipulation of tuberization by altering nutrient supply in hydroponics is likely to be indirectly linked. Factors including either accumulation of nutrients in the shoot system, remobilisation of stored nutrients or altering other physiological processes may be involved.

Plant water uptake rate decreased from early to late developmental stages. The high water uptake rate in early stages might be due to higher transpiration rates in younger leaves compared to mature leaves and stem tissue. During the early stage, the ratio of young leaves to mature leaves and stem tissue was large, so the rate of water loss was high. As the plant grows, the ratio of young leaves to mature leaves and stems declines, and rate of water loss per unit plant weight decreases.

The water uptake rates measured in this study did not agree with Nelson and Hwang (1975) who found that the weekly measured transpiration rate peaked at 21 days after emergence and then slowly declined throughout the rest of the experiment.

Uptake rate of water and all nutrient elements but $SO_4^{2-}$ and $H^+$ decreased with increasing plant fresh weight. Plants with lower fresh weight have higher uptake rate while plants with higher fresh weight have lower uptake rate. This trend of nutrient uptake was not caused by nutrient availability in nutrient solution, as the large volume of the solution reservoir ensured that concentrations of nutrient elements removed relatively constant over the duration of each uptake measurement.

Nutrient elements are taken up by plant roots from solution within the water flow, hence plant water uptake generally influences nutrient uptake. The relationship between plant water and nutrient uptake has been reviewed (Viets, 1972; Hsiao, 1973). Long periods of transpiration had little effect on uptake by roots in low external solution concentrations, but had a significant effect when the external concentrations were high (Kramer and Boyer, 1995). The nutrient concentrations were relatively high in the nutrient solution in this study. The amount of $NO_3^-$, $PO_4^{3-}$, $K^+$ and $H^+$ taken up by plants showed high correlation with water uptake, while $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ were not correlated with water uptake. The uptake of nutrients into plant roots is mediated by membrane transport proteins embedded in the plasmalemma. The specificity of these transporter activities, permeases and the mechanisms by which they are regulated, all regulate the entry of inorganic ions into the symplasm of plant roots.

Application of the growth retardant paclobutrazol decreased plant water uptake at the stolon elongation (stage 2) and tuber setting (stage 4) stages, and uptake rates of $NO_3^-$ and $K^+$ at the stolon elongation stage and tuber setting stage were also decreased. Paclobutrazol promoted tuberization in this study, which is similar to previously reports (Bandara and Tanino, 1995; Simko, 1993), but the effect did not appear to be mediated through changes in nutrient uptake rates, because at the swelling stage (stage 3), uptake rates of all measured nutrient elements from paclobutrazol treatment and control were similar.

Plant water uptake is strongly influenced by the environment, and in particular irradiance (Sawas and Lenz, 1995) and water vapor pressure deficit (Gracie, 2002). In this study, $NO_3^-$, $PO_4^{3-}$, $K^+$ and water uptake were correlated with photosynthetically active radiation. $H^+$ uptake displayed a positive correlation with temperature. While uptake rates were only recorded in this study in cloud free days, variation in irradiance between days and seasons is likely to have contributed to some of the variability in the data.

# CHAPTER 5

# WATER, GROWTH AND TUBER INITIATION

## OUTLINE

*The results of experiments reported in Chapter 3 and Chapter 4 revealed differences in stolon growth rate and water uptake rate between plants in inductive and non-inductive conditions. In Chapter 3 it was found that the initiation of tubers in crops occurred when the overall growth rate of stolons was high. When individual stolons were examined, tuber initiation was preceded by a period of rapid stolon growth. A close relationship was found between the rate of stolon elongation and the initiation of stolon swelling and subsequent tuber growth. In Chapter 4, reduced water uptake rate was reported in crops grown in conditions that delayed tuber initiation. Chapter 5 of the thesis reports on experiments investigating the effect of plant water status on growth of stolons and tubers under inductive and non-inductive environmental conditions. The chapter includes a review of the literature on water relations in potatoes and two subchapters detailing experimental work undertaken in the project. Chapter 5A describes the development of a novel non-destructive technique to record stolon and tuber growth, and the use of the technique to record diurnal growth of stolons and tubers. Chapter 5B describes the diurnal pattern of plant water status in stolons and tubers under different environmental factors. The timing and rate of stolon and tuber growth, and corresponding changes in plant water status are discussed.*

# INTRODUCTION

Water availability is one of the most important factors influencing potato crop growth and yield, and it is therefore not surprising that much research attention has been given to irrigation management in the crop.

The potato crop has a high water requirement for rapid growth and high yields, and many studies have demonstrated the yield decline in field crops associated with periods of water deficit. Research on irrigation management has addressed the physiological basis of drought sensitivity in potato and the agronomic relationships between timing and rate of water supply and tuber yield and quality. In addition to total yield, water availability is known to influence tuber number. The physiological basis of this relationship has not been described in as much detail as the overall yield effect, and many contradictory reports can be formed in the literature.

# LITERATURE REVIEW

## Water Availability and Crop Yield

Much of the research on water availability and crop yield in potatoes has utilized field and pot irrigation treatments to examine tuber yield and quality. Most early irrigation experiments concentrated on the relationships between water stress and tuber yield and quality. Moisture stress was demonstrated to decrease potato yield (Struchetemeyer, 1954; Jones and Johnson, 1958; Martin and Miller, 1983; Hang and Miller, 1986; Eldredge *et al.*, 1992; Shock *et al.*, 1992; Stark and McCann, 1992; Thimmegowda and Devakumar, 1993), reduce tuber number and weight (Thimmegowda and Devakumar, 1993) and increase the percentage of malformed tubers (Robin and Domingo, 1956; Nichols and Ruf, 1967; Painter and Augustin, 1976). Only a few contradictory results have been published, for example, Painter and Augustin (1976) reported that total tuber yield was not significantly affected by water stress. A higher root water uptake and tuber yield from mulched plants compared with unmulched treatments were reported by Bharat *et al.* (2000),

implying that high soil moisture increased tuber yield. In a series of long term field studies, Haverkort *et al.* (1990) found that the relationship between rainfall and tuber number was linear during the 12 seasons examined. The weight of evidence therefore indicates that potato tuber formation and growth are sensitive to water availability.

The effect of water stress on tuber yield depends on variety (Wilcox and Ashley, 1982) and plant development stage (Thimmegowda and Devakumar, 1993). Reduced tuber yield was evident in stressed cultivar Superior and Russet Burbank plants, but CA02-7 and Atlantic exposed to the same stress exhibited higher yields than control plants (Wilcox and Ashley, 1982). Exposure of crops to drought late in development was more detrimental than early drought on potato yield (Struchetemeyer, 1954; Jones and Johnson, 1958). In addition, tuber yield was reduced by soil moisture stress during stolon elongation (by 13 %), tuberization (41 %) and tuber development (47 %) (Thimmegowda and Devakumar, 1993).

Water stress to different part of the underground zone may result in different effects. Struik and van Voorst (1986) demonstrated that drought to roots had no effect on the numbers of stolons or tubers despite a yield reduction, whereas drought to stolons enhanced tuber initiation but only caused a minor reduction in yield. This suggested that water deficits around the stolons should be distinguished from deficits within the root zone as a whole.

The majority of studies on potato crop irrigation management have concluded that yield reductions from water stress resulted principally from reduced tuber size rather than tuber number. Increased tuber yield in crops under irrigation was due to an increase in tuber size rather than in tuber number (Fulton and Murwin, 1955) and tuber growth rate was drastically reduced when the available moisture level dropped. Exposure of potato plants to short periods of moderate water deficit retarded or stopped tuber growth (Moorby, 1970; Moorby and Milthorpe, 1975). The decrease in tuber growth under conditions of water deficit has been ascribed to direct effects on tuber growth and photosynthesis but not to interference with photosynthate transport. Low leaf water potential has been shown to result in a

decrease in translocation that was proportional to the decline in net photosynthesis (Munns and Pearson, 1974).

Shimshi *et al.* (1983) and Munns and Pearson (1974) reported a decrease in photosynthesis under water stress. The decrease in photosynthesis under water stress was accompanied by the accumulation of soluble sugars in the leaf. Basu *et al.* (1998) also found that an increase in the amount of total soluble sugars accompanied a decline in photosynthesis in water stressed greenhouse potato plants. However, changes in chlorophyll fluorescence transients were not closely related to changes in leaf water potential (Jefferies, 1992). The lack of a close relation between changes in chlorophyll fluorescence transients and leaf water potential may reflect that water potential can vary widely both throughout the day and from day-to–day, but chlorophyll fluorescence transients may not be sensitive to short-term environmental perturbations (Jefferies, 1992).

A number of studies have been performed in an attempt to define the critical threshold levels of plant water stress, beyond which the plant is adversely affected. Among these studies, stomatal closure was noticed when the leaf water potential was between -0.3 and -0.4 MPa (Campbell *et al.*, 1976). Leaf and tuber growth ceased at a water potential of -0.5 MPa (Gandar and Tanner, 1976). However, leaf water potentials in drought potato crops rarely falls below about -1.0 to -1.5 MPa (Rutherfoord and de Jager, 1975; Gandar and Tanner, 1976; Shimshi *et al.*, 1983; Stark and Wright, 1985; Jefferies, 1989).

An enhanced sensitivity to water stress at the tuber initiation phase was also reported (Coleman, 1988). Few other studies have examined water relations during tuber initiation. Maintaining high moisture levels has been shown to promote early tuber set (Bradley and Pratt, 1955), while water stress before tuberization resulted in reduced tuber number (MacKerron and Jefferies, 1986; Haverkort *et al.*, 1990; Shock *et al.*, 1992) and total tuber weight (Shock *et al.*, 1992). The reduction in tuber number per stem appeared to be caused mainly by a reduction in the number of stolons per stem when water stress occurred before tuberization; later water stress had no effect on tuber number (Haverkort *et al.*, 1990). Pereira and Pedras (1995) found that the highest water consumption occurred during stolon formation

and tuber initiation. Isolating the stolons in a low relative humidity environment delayed tuber set but increased the number of tubers per plant (Cary, 1986).

## Water Relations and Growth

Plant growth rate is linked to plant water status. Gandar and Tanner (1976) found that leaf elongation rate declined as leaf water potential decreased, and the leaf and tuber growth ceased at water potential of –0.4 to –0.5 MPa. Eguchi *et al.* (1998) also suggested that growth rate was closely correlated with changes in leaf water potential. The growth rate of beetroots, carrots and radishes was affected more by relative humidity than by temperature, and was always higher in the evening and at night when the relative humidity was higher (Kozlov, 1989). An increase in relative humidity from 25 % to 95 % caused a three- to four-fold increase in the rate of hypocotyl elongation (McIntyre and Boyer, 1984).

Plant growth involves cell division and cell enlargement, both of which require water uptake and some solute transport. The sensitivity of cell division to changes in tissue water potential (Hsiao, 1973) and water uptake as the driving force for cell enlargement have been demonstrated (Boyer, 1968; Cleland, 1971, 1977). Baker and Moorby (1969) reported that the amount of water entering or leaving the tubers when the plant leaves were exposed to dark or light was approximately 10 per cent of the tuber fresh weight. When water uptake was restricted, leaf water potential decreased and leaf and shoot expansion ceased (Raviv and Blom, 2001). Increasing relative humidity reduced transpiration, and increased the water potential and turgor of growing cells, which in turn caused an increase in the rate of hypocotyl elongation (McIntyre and Boyer, 1984). The rate of wheat leaf expansion was also proportional to leaf turgor potential (Li *et al.*, 1991).

The maintenance of turgor pressure as the plant water potential declines is crucial for cell expansion, for growth and for many of the associated biochemical, physiological and morphological processes (Hsiao, 1973; Hsiao *et al.*, 1976). When water potential declines, the process contributing to the maintenance of turgor

includes lowering osmotic potential due to either a naturally high solute concentration, an accumulation of solutes, or high tissue elasticity. There are two aspects of solute accumulation (Jones *et al.*, 1981). Many species accumulate a high concentration of solutes regardless of their level of water stress. These species should be able to maintain turgor and normal cellular function at low tissue water potential. However, some species with naturally low solute concentration have the capacity to accumulate additional solutes in response to water stress and to achieve some measure of osmotic adjustment. Accumulation of organic solutes, principally the amino acid proline, has been demonstrated in potato in response to water deficit (Bussis and Heineke, 1998). This osmoregulation permitted continued growth and photosynthetic activity in the stressed plants (Bussis *et al.*, 1998)

## Plant Diurnal Growth Pattern

Plant water status, and tissue growth rates vary with environmental conditions. These variations include diurnal cycles associated with light/dark phases each day. Diurnal changes in the fruit expansion have been studied in citrus (Elfving and Kaufmann, 1972), apples (Higgs and Jones, 1984; Tromp, 1984) and tomatoes (Johnson *et al.,* 1992; Kitano *et al.* 1996). In general, fruit growth does not occur continuously throughout the day and night, but follows a diurnal pattern of expansion and contraction. Expansion occurs primarily at night while contraction occurs during the daylight hours. For example, both terminal and lateral fruitlet growth rates of Golden Delicious apples decreased approximately one hour after sunrise and growth rate increased between 14:00 and 15:00 on clear sunny days (Bergh and Cloete, 1992).

Diurnal fluctuations in growth rate in underground organs have also been reported. Baker and Moorby (1969) showed that the increase in potato tuber fresh weight occurred predominantly in the night. The greatest increase in tuber diameter expansion of potato (Eguchi, 2000) and sweet-potato (Eguchi *et al.*, 1997) occurred during the early night, and transient contraction of the tuber occurred at the beginning of the light period. Stark and Halderson (1987) showed that the potato

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tuber followed a diurnal pattern of expansion and contraction, but these authors found that the expansion occurred primarily during the late night to early morning hours.

A number of methods have been developed to study diurnal growth pattern in tubers. Diurnal changes in tuber volume were first demonstrated by Macmillan (1891). Since that time, different methods have been developed for measuring plant diurnal growth. The electric weighing system (Baker and Moorby, 1969), the linear voltage displacement transducer (LVDT) system (Stark and Halderson, 1987; Gracie, 2002) and more recently, the laser micrometer (LM) (Eguchi *et al.*, 1997; Eguchi, 2000) have been used successfully.

## Diurnal Pattern in Plant Water Status

Fluctuations in plant water status are thought to drive the diurnal fluctuations in plant tissue growth rate. Gandar and Tanner (1976) measured diurnal patterns in leaf growth and water potential and showed that leaf growth rate ceased when water potential declined during the morning, while leaf growth recommenced as leaf water potential increased from midday to nighttime. Johnson *et al.* (1992) studied water relations and fruit growth in tomatoes under different levels of radiation. Under low radiation (80 $W.m^{-2}$) fruit diameter continued to increase both during the day and night, but with the nighttime rate being significantly higher. Fruit osmotic potential varied between –0.65 and –0.95 MPa under low radiation, while under high radiation (> 400 $W.m^{-2}$) the osmotic potential varied between –1.25 and –1.40 MPa and fruit diameter increased rapidly during the night but decreased significantly during the day.

The above studies suggest that water plays a key role in the diurnal pattern of plant growth. The importance of water on diurnal growth of potato tuber can be seen from the results of Stark and Halderson (1987), in which the expansion of potato tuber occurring during the late night and early morning, the period associated with maximum turgor recovery (Kramer, 1983). Shortly after sunrise, tubers began to

contract and reached a minimum circumference during the afternoon hours. The increase in cell volume could be considered equivalent to the amount of water uptake by the cell, because water must flow into cells during cell expansion, and water occupies more than 80 % of fresh weight in plant cells.

While diurnal growth patterns in tubers driven by cycles of increasing and decreasing plant water potential have been demonstrated, the diurnal pattern of stolon growth has not been reported. As the results presented in the previous chapters have indicated a relationship may exist between stolon growth rate and timing of tuber initiation, diurnal patterns in stolon growth may provide clues to the timing and early events of tuberization. A link between stolon growth rate and tuber initiation may also explain the effect of water deficits on tuber number in field grown crops. Detailed investigation of the diurnal patterns of potato plant water relations and stolon growth rate were therefore warranted in this project.

# CHAPTER 5A

# DIURNAL PATTERN OF STOLON AND TUBER DEVELOPMENT

## OUTLINE

***The timing and rate of tuber initiation was analysed in Chapter 3B. One important finding was that the elongation rate of stolons peaked 1 - 2 days prior to swelling, suggesting a relationship between rate of stolon elongation and tuber initiation. More detailed studies of the growth rate of stolons and tubers are described in this chapter. A novel non-destructive method for measuring diurnal pattern of plant growth was developed and validated. High-resolution webcams and low intensity lights controlled by a timer system were used to acquire sequences of images of developing stolons and tubers. The method was successfully applied to measure the diurnal growth pattern of stolons and tubers. The rate of stolon elongation and tuber expansion, and the timing of swelling initiation during the diurnal cycle were obtained, and differences in diurnal patterns with growth seasons were discussed.***

## INTRODUCTION

The results of the morphological study presented in Chapter 3 indicated that timing of tuber initiation was related to the rate of stolon elongation. The initiation of swelling in crops generally occurred a few days after stolon growth rate reached a peak (Chapter 3B). These results were based on measurements of stolon length made once a day with a ruler, and were thus a crude estimate of stolon growth rate.

Study of the relationship between stolon growth and tuber initiation requires techniques to more accurately and frequently measure stolons and tubers. Many previous studies have shown that plants follow a diurnal growth pattern, with slow growth or contraction at about midday and rapid growth from late afternoon to early evening (Baker and Moorby, 1969; Elfving and Kaufmann, 1972; Higgs and Jones, 1984; Tromp, 1984; Stark and Halderson, 1987; Johnson *et al.*, 1992; Kitano *et al.*, 1996; Eguchi, 2000). No reports of diurnal patterns in stolon growth have been published.

Comparing the reported systems for diurnal measurement of plant growth, the image recording systems appear more suitable for assessing stolon and tuber growth than the LVDT system as both stolon length and tip diameter which reflects the degree of swelling can be measured. In addition, the relatively rapid elongation rate of stolons does not require the high sensitivity of measurement provided by the LVDT system. The image recording system would allow assessment of growth rate of stolons and the timing of swelling using a single system.

Using a digital image capture system, the diurnal growth pattern of stolon and tuber under different growth seasons was studied. The diurnal pattern of stolon growth rate and tuber expansion rate, and the concurrent rate of stolon elongation and expansion were demonstrated. The timing of swelling initiation in a diurnal cycle was identified.

# METHODS AND MATERIALS

The diurnal growth pattern of stolons and tubers were measured using a webcam system (system description and usage are detailed in Chapter 2).

Only actively growing stolons and tubers of normal appearance were used for measuring the diurnal growth pattern. Webcams and light sources were set about 10 cm above each stolon/tuber. A high precision ruler was placed next to the stolon/tuber to provide a scale to convert the length of stolon or the diameter of tuber from pixels to millimetres. The lights were controlled by a timer and were switched on for 1 minute at hourly intervals. The webcams were set to capture images when changes in light level were detected.

Stolon diurnal growth pattern was measured in crops grown in January-February, May-June, July-August and November-December 2002. All stolons were from different plants. Measurements were made for a total of three stolons and within each crop all measurements were made on the same day. All measurements were made in crop growth stage 2 (stolon elongation stage). The growth of individual stolons over three consecutive days was studied in crops in March-April and September-October 2002. Six stolons in each crop were examined.

Tuber diurnal growth pattern was measured in a crop grown in March-April and May-June 2002. Measurements were made for a total of 6 tubers. The variation in diurnal growth pattern of the same tuber between days was measured for three consecutive days in crops grown in March-April, May-July and July-August 2002. Six tubers in each crop were examined. All tubers for measurement were between 5 and 10 mm in diameter.

To detect the timing of tuber initiation in a daily cycle, three stolons from a crop grown in July-August 2002 were examined over a 7 day period concluding when swelling of the stolon tip was observed. Stolons selected for study were those

considered likely to undergo swelling in a few days, based on the characteristic colour change described in Chapter 3.

Images of stolons and tubers were collected every hour. Image analysis involved calculation of stolon and tuber length and diameter based on the number of pixels corresponding to stolon and tuber tissue in the images. Validation of the system through repeated measurements of the object of known size demonstrated accuracy to be ± 0.05 mm. Stolon and tuber diurnal growth patterns were then plotted as both absolute increases in size with time and as growth rates. Patterns of absolute increases in size were plotted every hour and growth rates were plotted every two hours. As the data were used to describe the growth pattern, no statistical analyses were performed.

# RESULTS

## Pattern of Stolon Elongation

A consistent pattern of stolon growth was recorded for all elongating stolons examined. Stolon elongation was most rapid from late afternoon to early or mid evening, followed by a period of slower elongation until mid morning. Stolon elongation ceased, or in some cases stolon contraction occurred, in the late morning to early afternoon period (Fig. 10).

Elongation rate of different stolons measured on the same day displayed significant variability. Both rapidly and slowly elongating stolons could be found in plants growing in the same hydroponic tray during the stolon growth stage (Fig. 10B). Diurnal patterns were most pronounced in rapidly elongating stolons.

The diurnal pattern of stolon elongation consisted of a slow or negative elongation rate at about midday, and a rapid elongation rate from about late afternoon to early evening in each of the different seasons (Fig. 11). The precise timing of periods of rapid growth and declining growth rate within the diurnal pattern differed between seasons. The commencing time of the rapid increase in stolon length varied from between 12:00 and 15:00 for plants grown in May-June and July-August, to 16:00 and 18:00 for plants grown in January-February and November-December. Timing of periods of rapid elongation was more uniform between stolons within each crop than between crops, although the elongation rate did vary considerably within crops. When mean values were calculated, the daily maximum stolon elongation rate varied between seasons. The highest daily maximum elongation rates in May-June, July-August, November-December and January-February were 1.75, 0.1, 0.46 and 0.14 mm/h respectively.

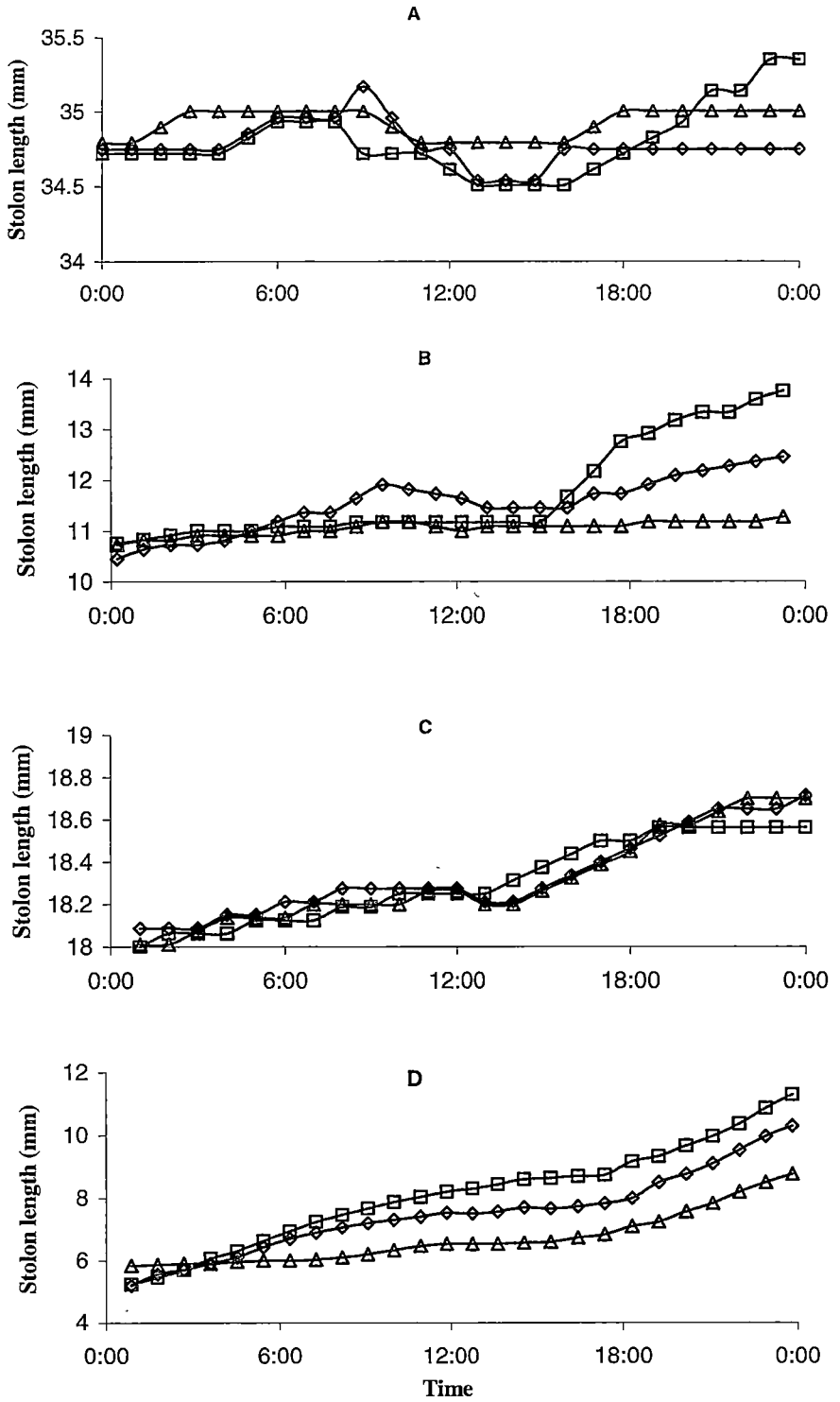


**Figure 10.** Diurnal pattern of stolon length measured on the same day from three different plants grown in January-February (A), May-June (B), July-August (C) and November-December (D) 2002. Different symbols showed different stolons. Data were recorded at stage 2.

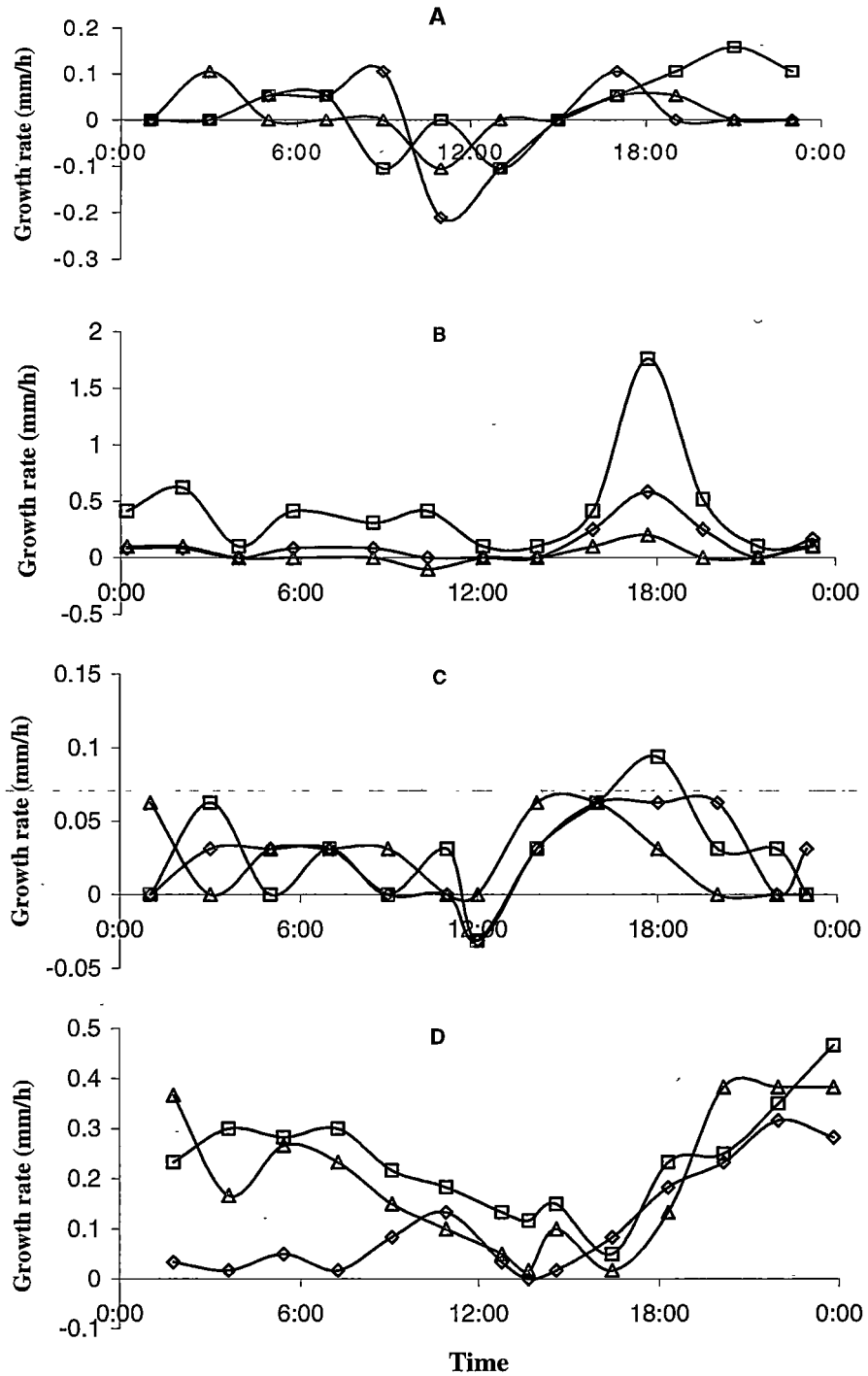


**Figure 11.** Diurnal stolon elongation rate measured on the same day in three different plants grown in January-February (A), May-June (B), July-August (C) and November-December (D) 2002. Data were recorded at stage 2.

Diurnal stolon elongation patterns were similar over consecutive days for individual stolons (Fig. 12). A slight increase in length or contraction in length occurred around midday, which was followed by a rapid elongation in mid to late afternoon. However, the elongation rate and precise timing of the rapid elongation phase varied between days. The stolon recorded in the March-April crop appeared to have ceased elongating at day 3 (Fig. 12A), but a small contraction commencing at 12:00 followed by elongation to return to its original length was still observed.

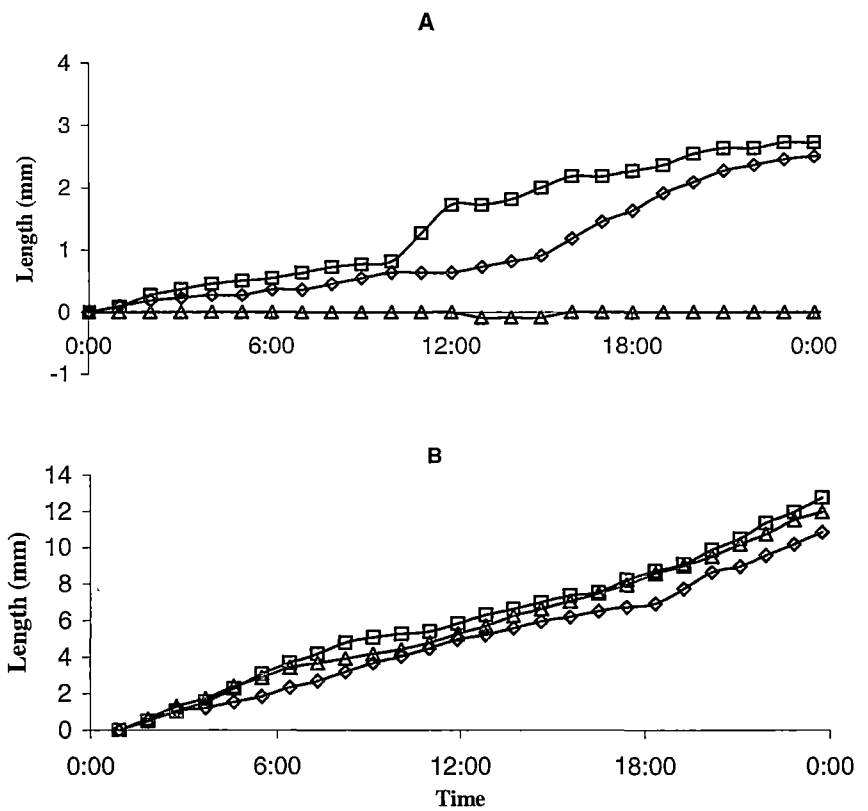


**Figure 12.** Diurnal length of the same stolon over consecutive three days in March-April (A) and September-October (B) 2002. Where day 1 is (◇), day 2 is (□) and day 3 is (△). Data were recorded at stage 2.

The elongation rate of individual stolons over three days displayed similar diurnal patterns in the March-April crop with low elongation rate at about midday and increased rate in the afternoon and evening (Fig. 13A). The size and timing of the peak in growth rate varied between days, with rates of 0.27 mm/hr at 16:00 from

day 1, 0.14 mm/hr at 19:00 from day 2 and 0.05 mm/hr at 17:00 from day 3 recorded. More rapid growth rates were recorded in the September-October crop (Fig. 13B), but the diurnal elongation patterns were less discernable. No obvious decline in elongation rate around midday was observed, but elongation rate did increase in all stolons in the late afternoon period, peaking at 20:00, 22:00 and 18:00 with values of 0.91, 0.86 and 0.61mm/hr for day 1, 2 and 3 respectively.

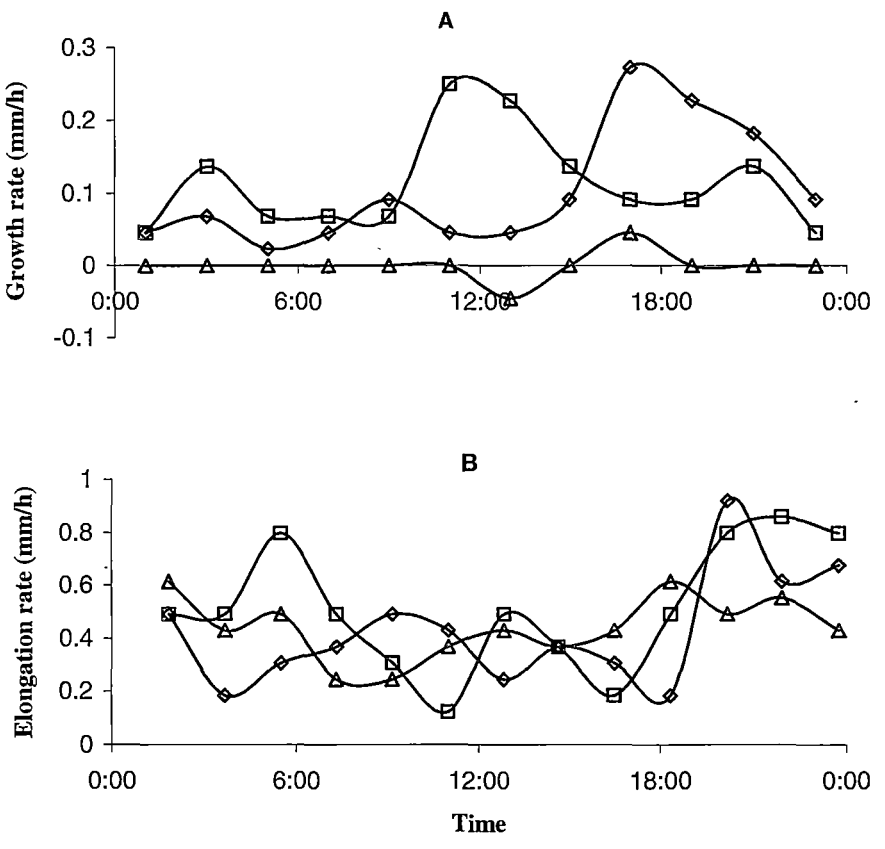


**Figure 13.** Elongation rate of the same stolon over three consecutive days in March-April (A) and September-October (B) 2002. Where day 1 is (◇), day 2 is (□) and day 3 is (△). Data were recorded at stage 2.

## Pattern of Tuber Expansion

Tuber diameter increased rapidly and continuously from midday to early morning, but increased more slowly from early morning to sunrise. At late morning to early afternoon, tuber diameter did not change or decreased slightly (Fig. 14).

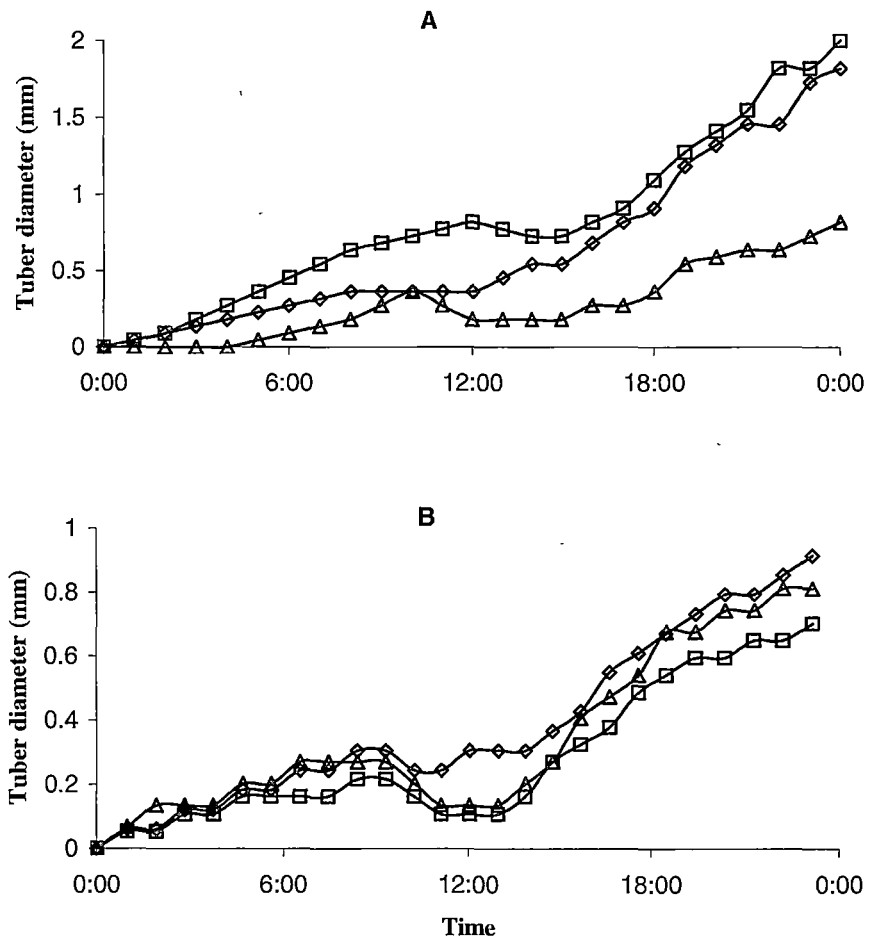


**Figure 14.** Diurnal change in tuber diameter measured on the same day from three different plants grown in March-April (A) and May-June (B) 2002. Data were recorded at stage 3.

The diurnal pattern can be seen more clearly when tuber expansion rate is plotted (Fig. 15). The change in diurnal tuber expansion in the March-April and May-June crops showed a similar pattern. Tuber expansion rate decreased at about midday, while rapid expansion rates were found from afternoon to early evening.

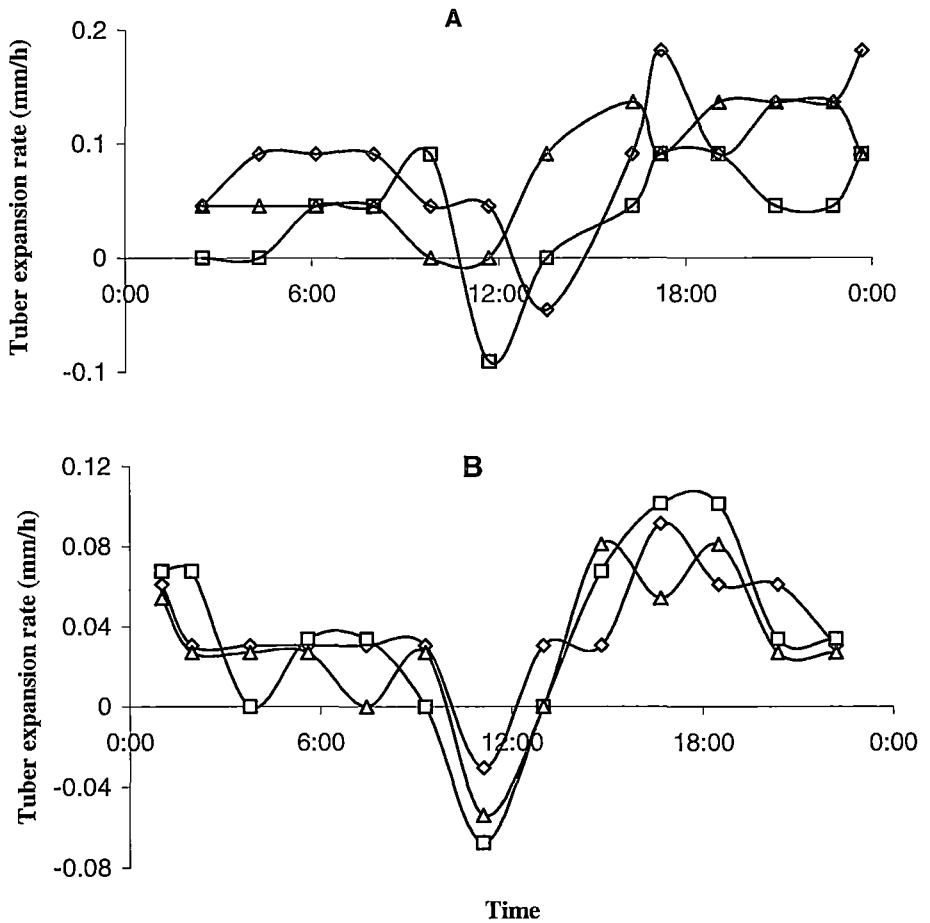


**Figure 15.** Diurnal pattern of tuber expansion rate measured on the same day in three different plants grown in March-April (A) and May-June (B) 2002. Data were recorded at stage 3.

The measurement of diameter of the same tuber over three consecutive days showed a similar pattern with a rapid expansion from afternoon to midnight and a slow expansion at midday (Fig. 16).

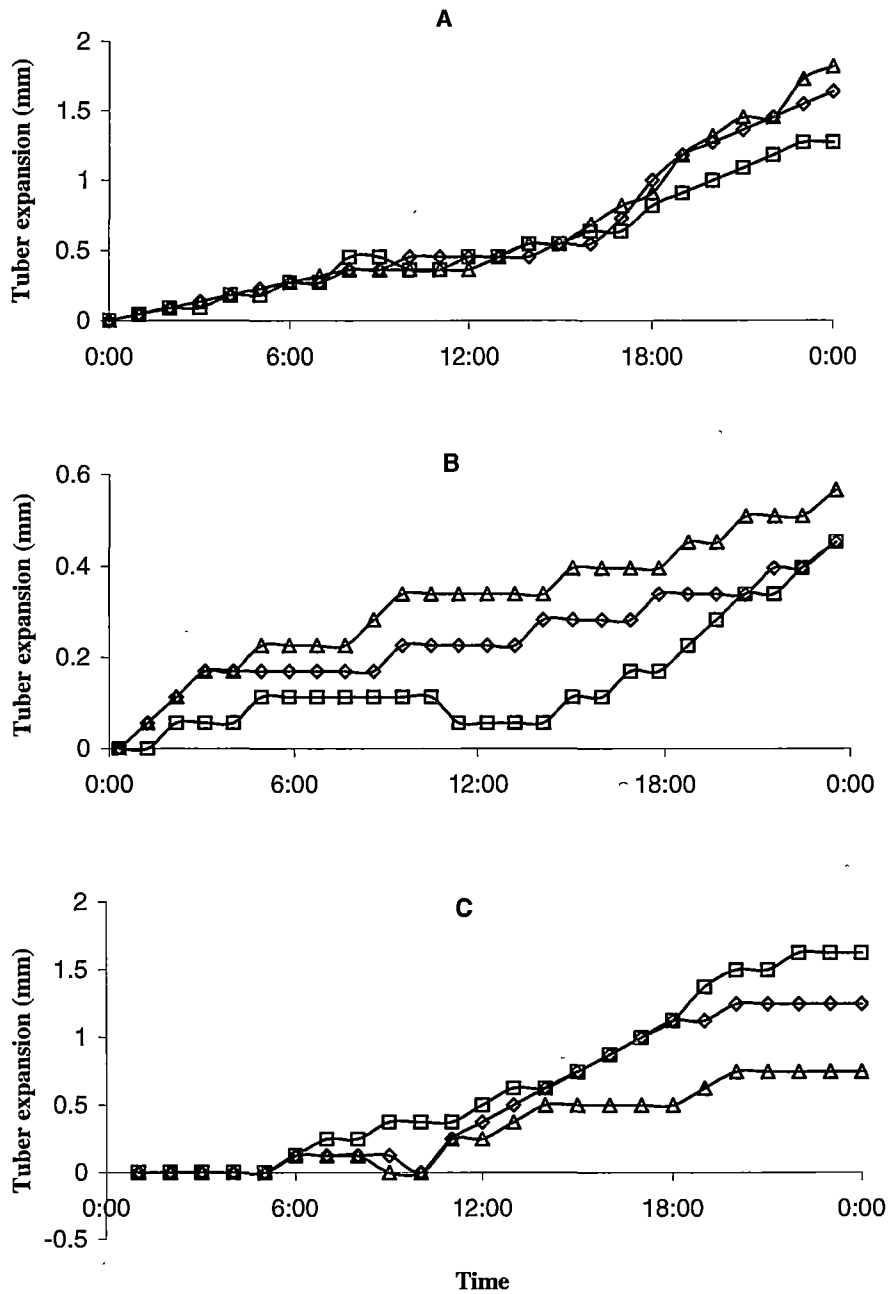


**Figure 16.** Change in diameter of the same tuber over three consecutive days in March-April (A), May-June (B) and July-August (C) 2002. Where day 1 is (◇), day 2 is (□) and day 3 is (△). Data were recorded at stage 4.

Tuber expansion rate in diameter over three consecutive days generally showed a similar pattern, with low expansion rates at midday, which were followed by high rates (Fig.17).

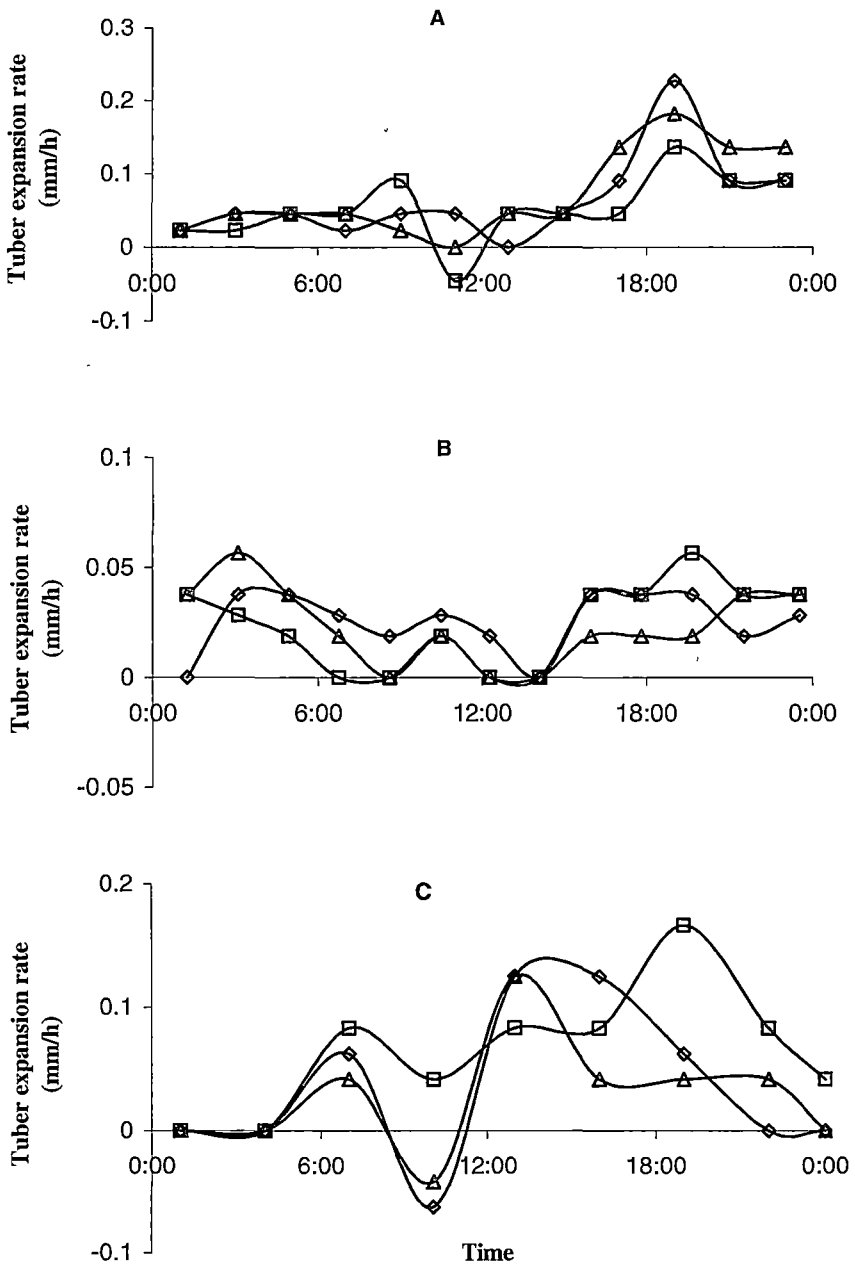


**Figure 17.** Diurnal pattern of tuber expansion rate from the same tuber over three consecutive days in plants grown in March-April (A), May-June (B) and July-August (C) 2002. Where day 1 is (◇), day 2 is (□) and day 3 is (△). Data were recorded at stage 4.

## Timing of Swelling

The timing of swelling of stolon tips was assessed by measurement of the ratio of stolon tip diameter to stolon diameter measured 10mm behind the stolon tip over a 7 day period. During the period three out of the six stolons examined commenced swelling. The commencement of swelling occurred on a different day for each of the three stolons. Changes in tip to stolon diameter ratio was plotted over a 3.5 day period, with starting point being 1 day prior to commencement of swelling (Fig. 18). The individual stolon plots thus do not represent the same 3.5 day periods. Swelling commenced at approximately 12:00, which was around the time that rapid growth of stolons was also observed (Fig. 11C). In elongating stolons, the ratio of stolon tip to stolon diameter varied only slightly over the 3.5 day period. The rapid increase in ratio of stolon tip to stolon diameter after swelling from afternoon to midnight was slow compared to that of the second or third day after swelling. At the commencement of swelling, the swelling tip expanded while stolon elongation continued. However, stolon elongation ceased on the second or third day after swelling, and at this time the increase in ratio of stolon tip to stolon diameter was most rapid (Fig. 19).



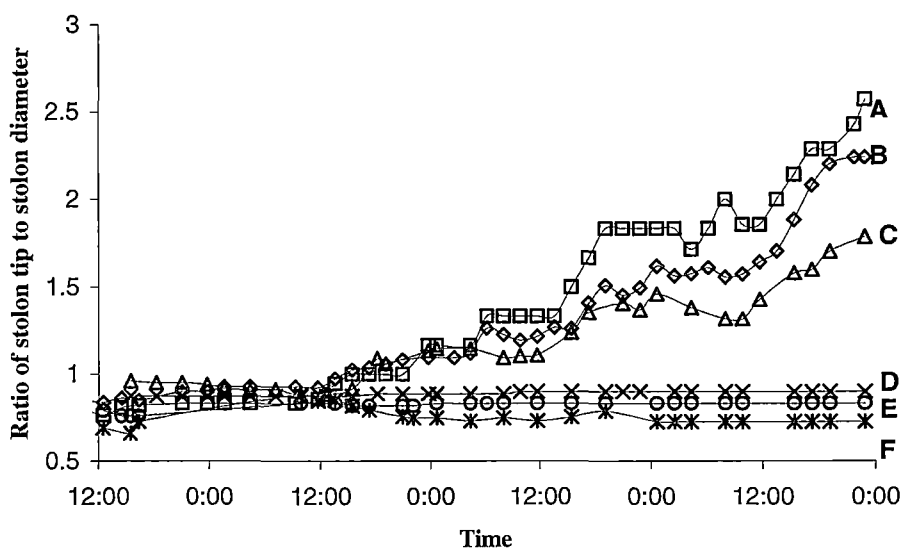


**Figure 18.** Ratio of stolon tip to stolon diameter (10 mm behind stolon tip) of swelling (A, B, C) and non-swelling (D, E, F) stolons measured from one day prior to swelling through to swelling. Data were from crops grown in July-August 2002.

Analysis of the longitudinal and radial growth rates of stolons around the time of swelling initiation clearly showed that swelling initiation was accompanied by a high stolon elongation rate. After swelling initiation, stolon elongation decreased. The highest elongation rate during swelling initiation was 0.28 ± 0.06 mm/h, but decreased significantly ($p < 0.05$) toward the second day. At the second day after swelling the highest elongation rate was only 0.08 ± 0.02 mm/h (Fig. 19). At the same time the maximum radial expansion rate did not changed significantly. The maximum radial expansion rate at the day of swelling and the second day after swelling was 0.08 ± 0.02 and 0.08 ± 0.01mm/h respectively.

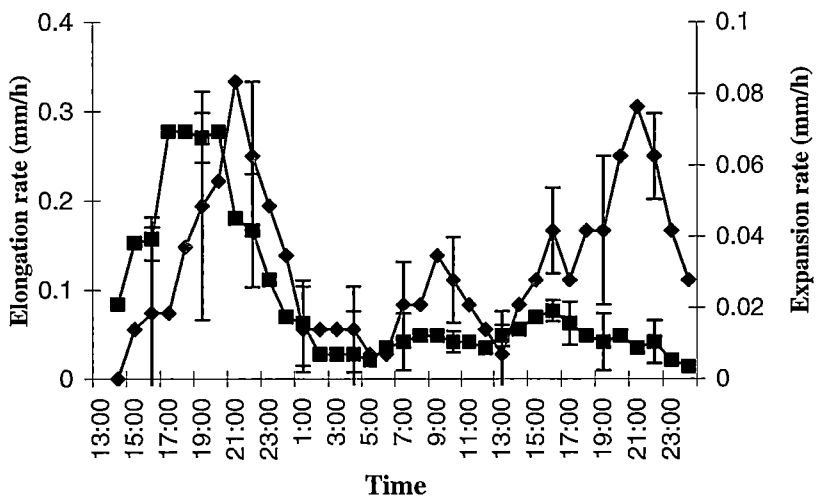


**Figure 19.** The rate of stolon elongation (■) and stolon tip expansion (◆) measured in the July-August crop 2002. Measurement started from the initiation of stolon swelling. Each value was an average of three stolons.

# DISCUSSION

Fluctuations in potato tuber growth have been observed during 24 hour cycles, with rapid growth in volume (Eguchi, 2000), diameter (Stark and Halderson, 1987) and weight (Baker and Moorby, 1969) during the late afternoon to early evening. Similar diurnal growth patterns have been described for many other plant species (Elfving and Kaufmann, 1972; Higgs and Jones, 1984; Tromp, 1984; Johnson *et al.*, 1992; Kitano *et al.*, 1996).

The diurnal pattern of growth of potato tubers described in this study was consistent with the observation of Eguchi (2000) and Stark and Halderson (1987), but more detailed observations of variability between tubers in timing and rates of expansion were also made. Rapid expansion in tuber diameter occurred from late afternoon to midnight, while tuber shrinkage commenced between midmorning and midday. Tuber expansion rate generally peaked during the afternoon and declined to low levels by midnight. Cessation in rapid tuber expansion by midnight was also reported by Eguchi (2000) in hydroponically grown plants, but continuous expansion through the night was reported in pot grown plants (Stark and Halderson, 1987). While differences in environment could explain the differences in timing of expansion growth, the difference in the duration of expansion could also be influenced by plant water relations. Plants grown in pots (Stark and Halderson, 1987) would be exposed to lower water potential in the root zone than hydroponically grown plants so these plants may require a relatively long time to recover water content after the transpirational losses. Hence the rapid tuber expansion would be expected to occur later and persist longer. The timing of rapid growth and the rate of the diurnal growth varied between tubers in the same tray. This is consistent with the report of Mares *et al.* (1985) and Schnieders *et al.* (1988) that tuber growth rate varied between plants and between tubers within a plant.

Stolon elongation rate displayed a similar diurnal pattern to tuber expansion rate, with very slow growth or in some cases shrinkage at midday, followed by a period of rapid elongation and then a prolonged period of slower growth. The diurnal

pattern of stolon elongation has not been previously reported in the literature. Differences in elongation rate between stolons on the same plant, although not described within a diurnal pattern, have been reported previously (Lovell and Booth, 1969).

The characteristic diurnal pattern of tuber expansion and stolon elongation fits that of previously described fruits (Elfving and Kaufmann, 1972; Higgs and Jones, 1984; Tromp, 1984; Johnson *et al.,* 1992; Kitano *et al.,* 1996), roots (Eguchi, 1997; Gracie, 2002) and tubers (Stark and Halderson, 1987; Eguchi, 2000). The potato may contain several stolons and/or tubers at different stages of development at any time. The differences in timing and rates of growth of different stolons and tubers on the same plant on the same day recorded in this study clearly demonstrate the involvement of internal regulatory systems in elongation and expansion growth. In addition the effects of environmental conditions was evident in the daily variations in timing and rates of growth.

Although the growth of stolons and tubers in different seasons displayed a similar characteristic diurnal pattern, growth rates varied between seasons, days and stolons. Differences in environmental conditions may explain some of these variations, particularly those between the same stolon/tuber on different days. Growth rate in plants has been shown to be influenced by humidity, with more rapid growth in higher humidity environments (McIntyre and Boyer, 1984; Kozlov, 1989). Higgs and Jones (1984) have shown the close relationship between fruit shrinkage and radiation levels, with no shrinkage at low irradiance and most growth occurring during the dark periods. Others reported that in a number of crops leaf growth rate was temperature dependent (Gallagher, 1979; Milford *et al.*, 1985 a, b). Physiological status of stolons must also play a significant role in determining elongation rate. Cessation of stolon growth could not be explained by environmental changes, while both seasonal and stolon variability noted in the study suggests endogenous regulation. This would also be consistent with the previous observation (Chapter 3B) that stolon growth was relatively slow at the early stolon stage but rapid at the late stolon stage, peaking a few days prior to swelling initiation.

Use of the webcam system allowed more detailed study of stolon elongation and swelling after tuber initiation than that reported in the earlier chapters, and lead to the observation that the initiation of swelling occurred before stolon elongation ceased. However, on the day following swelling initiation, stolon growth rate declined to negligible levels while the expansion rate of the stolon tips increased. It was clear in this study that the first swelling was concurrent with stolon elongation, and that the cessation in stolon elongation was a response to the initiation in radial expansion at the stolon tip.

The initiation of stolon swelling was only observed to occur in mid to late afternoon following a period of low stolon elongation. This was the same point in the diurnal pattern where rapid stolon elongation and tuber expansion occurred. This suggested that the initiation of stolon swelling was regulated by the same factor driving the rapid increase in elongation and expansion growth in stolons and tubers, following the daily decrease in growth rate around the middle of the day. The commencement of radial expansion of the stolon tip, the first morphological sign of tuber initiation, was demonstrated to occur at a point in the diurnal growth pattern.

This first report of the precise timing of the initiation of swelling is an important and exciting finding in tuberization research. The timing was linked to the diurnal growth pattern, so more detailed examination of this aspect of potato growth is warranted. Although stolons and tubers showed a similar growth pattern, the timing of the rapid growth in the diurnal cycle and the maximum daily growth rate varied greatly. The initiation of stolon swelling was accompanied by stolon elongation. The rate of stolon elongation decreased only after swelling initiation but expansion rate increased. While this finding offers new ideas in understanding the initiation of tuber development, further research on the factors driving the diurnal growth pattern is required.

# CHAPTER 5B

# DIURNAL PATTERN OF WATER RELATIONS

## OUTLINE

***The description of the diurnal pattern of stolon elongation and tuber expansion reported in Chapter 5A included the first documented evidence that stolon swelling commenced at a specific time of the day. The timing of stolon swelling, as well as the peak in stolon elongation and tuber expansion rates in the diurnal cycle, suggested that the initiation of tuber development was linked to processes during rapid tissue expansion. Diurnal cycles in plant water relations have previously been shown to be associated with diurnal growth cycles in plants and therefore a series of experiments was undertaken to examine the diurnal pattern of water relations in potatoes during tuberization. This chapter describes the diurnal pattern of water status in leaves, stolons and tubers under different environmental conditions. The relationships between plant growth rate and water potential, osmotic potential and turgor are discussed.***

## INTRODUCTION

Water is especially important for potato crops, as potatoes are highly sensitive to water stress. When the plant was only subjected to slight water stress, potato stomata closed (Grander, 1975; Campbell *et al.*, 1976). Water stress increased leaf diffusive resistance and decreased transpiration (Wilcox and Ashley, 1982), reducing further water loss from plants. As a result, plant water uptake and water usage decreased. In addition to altering the pattern of water uptake and stomatal closure, water stress has been shown to decrease both the rate of tuber initiation (Llewelyn, 1963; Slater and Goode, 1967) and tuber development (Moorby, 1970; Fulton and Murwin, 1955). The effect of water stress on tuber initiation has

received less attention than effects of other environmental factors, and some contradictory results have been reported. Water shortage during tuberization was reported to reduce (Krug and Wiese, 1972), have no effect on (Jefferies and MacKerron, 1986) or even increase tuber number (Cavagnaro *et al.*, 1971). Sufficient evidence exists to justify further examination of water relations during tuberization.

Previous studies of potato tuber diurnal expansion patterns have documented rapid expansion in the afternoon, with a preceding period of tuber shrinkage (Eguchi, 2000; Stark and Halderson, 1987; Baker and Moorby, 1969; Chapter 5A). The diurnal measurement of tuber weight and carbohydrate movement into tubers suggested that the rapid growth period was mainly caused by an increase in tuber water content rather than by movement of photoassimilate into tuber (Baker and Moorby, 1969). Similarly, Johnson *et al.* (1992) provided direct evidence that tomato (*Lycopersicon esculentum*) fruit growth was closely linked to the movement of water into the fruit. The timing of the phase of tuber shrinkage appeared to correspond to the time of the day where leaf water potential would be expected to have declined to a relatively low level. The diurnal change in water potential in potato leaves and tubers is similar to that of other plant species, decreasing from early morning to midday but increasing from midday to nighttime (Gandar and Tanner, 1976; Campbell *et al.*, 1976). When the results of studies of diurnal pattern of tuber growth rate and plant water potential are considered together, it can be concluded that when water potential decreased from early morning to midday, growth rate decreased, and when water potential increased from afternoon to nighttime, growth rate increased. It has also been reported that sweet-potato tuber growth rate was closely correlated with changes in leaf water potential (Eguchi *et al.*, 1998). However, the contribution of osmotic potential and turgor to this pattern has not received research attention. An understanding of diurnal plant water status and tuber growth requires study of all components of leaf and tuber water status.

Plant growth is mainly driven by turgor pressure. Bunce (1977) found a linear relationship between leaf elongation rate and turgor in soybean in different environments. Turgor was closely associated with relative humidity. Increasing the relative humidity from 25 to 95 % caused a marked increase in the water

potential and turgor of the growing cells and led to a three- to four-fold increase in the rate of elongation of the hypocotyl (McIntyre and Boyer, 1984). A study of environmental factors on tuberization contained evidence that the relative humidity must be high enough around individual stolons for tuber initiation to occur. No tubers developed on stolons grown in dry environment, whereas tubers only occurred in stolons grown in wet conditions (Cary, 1986).

Osmotic potential plays a more important role in plants. A small decrease in osmotic potential may be accompanied by a large decrease in turgor and water potential (Kramer, 1969). Growth rate of maize leaf was increased when the osmotic potential of the growing region was reduced by supplying K to the roots or directly to the surface of the stem whatever the relative humidity (McIntyre and Boyer, 1984). Organic materials such as soluble sugars play an important role in plant tissue osmotic potential. The low rate of stolon growth and delay of tuber initiation under high temperature in summer may be due to the low level of osmotically active organic materials in the underground tissue. The translocation of organic materials is temperature dependent. Translocation increased continuously to approximately 30 °C, when temperature was higher than 30 °C translocation reduced (Hull, 1952).

This chapter investigated plant water status in leaves, stolons and tubers over the diurnal cycle to analyse the relationship between plant water status and tuber initiation. Water potential, osmotic potential and turgor were examined in stolons and tubers under inductive and non-inductive conditions. The results were discussed with emphasis on the possible role of osmotic potential and turgor in tuber initiation.

# METHODS AND MATERIALS

Leaf water potential was measured in crops grown in November-December 2001, January-February, May-June, July-August, September-October and November-December 2002. Stolon water relations were studied in plants grown in January-February and November-December 2002. Tuber water relations were investigated in plants grown in September-October 2002.

A preliminary series of measurements of water potential in leaves, stolons and tubers every three hours over three separate 24-hour periods was conducted in plants grown in November-December 2001. The measurement revealed that rapid changes in water status occurred over the diurnal period. As has been reported for other species, water potential declined from predawn to midday and rose rapidly in the afternoon. A gradual increase in water potential occurred at night, with plant and soil water potentials reaching equilibrium before dawn. Measurement of water status at predawn, midday and dusk could generally describe the main changes during a day. Hence in this study the measurement of plant water status was carried out at predawn, midday and dusk. In order to investigate the effect of weather conditions on leaf water potential, measurements of midday leaf water potential were undertaken in January-February 2002 over three consecutive sunny days and three consecutive days with different weather conditions including sunny, rainy and cloudy days.

Water status measurements included in the study were total water potential, osmotic potential and turgor potential. The total water potential of stolons, leaves and tubers was measured using a pressure chamber (Scholander *et al.*, 1965; Gandar and Tanner, 1976). Tuber water potential was measured by suspending tubers in the chamber on a 1-2 cm stolon. Tuber endpoints were determined the same way as leaf and stolon endpoints. Plant samples were measured immediately after being excised. Care was taken when measuring water potential that pressure did not exceed the point at which xylem sap reached the cut surface of the tissue. After measuring total water potential, stolon tip/tuber samples were sealed in 1.5 ml centrifuged tubes and were snap frozen in liquid nitrogen and stored at –20 °C

until required for analysis. Samples were thawed at room temperature for approximately 2 hours and the cell solution extracted by centrifugation at 12000 rpm for 5 mins. The osmotic potential of the extracted solution was measured with a Wescor Model 5100C vapor pressure osmometer (Wescor Inc., Logan, UT, USA), similar to Patrick (1984). Turgor was calculated as the difference between water potential and osmotic potential measured on the same sample.

The effects of inductive short days (SD) and non-inductive long days (LD) treatments were studied in plants grown in January-February 2002. Six hydroponic trays of plants were grown for a total of 35 days, at which point plants had developed stolons but had not reached the swelling stage. Three trays of plants were then moved into three separate growth tunnels. The tunnels consisted of motorised trolleys, controlled by timers, which moved the trays between insulated lightproof chambers and the glasshouse. The timers were set to move the plants into the chambers at 18:00 and back into the glasshouse at 10:00, giving plants a photoperiod of 8 hours. The remaining three trays were exposed to natural photoperiod, from about 6:00 to 20:30, providing a photoperiod of about 14.5 hours. Water status was measured at 5:00-6:00 (predawn), 12:00-13:00 (midday) and 17:00-18:00 (dusk) on each of three consecutive days.

The effect of paclobutrazol (PBZ) application to the crop on potato plant water status was studied in November-December 2001. Paclobutrazol (450 mg/L of PBZ plus 0.12 % V/V surfactant Cittowett) was applied when all plants had developed stolons but had not reached the swelling stage. The solution was sprayed onto the foliage of plants in three trays, with approximately 75 ml applied per plant. The volume applied was sufficient to wet all leaf surfaces. For the control treatment, distilled water was sprayed onto another three trays of plants. Three days after treatment, leaf water potential, stolon water potential and stolon osmotic potential were measured at 5:00-6:00 (predawn), 12:00-13:00 (midday) and 17:00-18:00 (dusk).

In order to study the relationship between water status of different organs, the concurrent measurement of water status in stolons, swelling tips and tubers from the same plant were undertaken in September-October 2002. A single stolon,

swelling tip and tuber was excised from each of 3 separated plants at 5:00-6:00 (predawn), 12:00-13:00 (midday) and 17:00-18:00 (dusk). Total water potential and osmotic potential of these samples were measured and turgor was calculated.

# RESULTS

## Water Potential in Leaves, Stolons and Tubers

The trend in water potential through the day was similar for leaves, stolons and tubers (Fig. 20). Water potential decreased from predawn to midday and increased from midday to dusk. The water potentials in the different organs at predawn and dusk were similar, but daytime water potentials varied. The minimum water potential was measured in the leaves and the highest water potential was in tubers greater than 2 cm diameter. The general order of water potential was: leaves < stolons < tubers less than 1.0 cm < tubers greater than 2.0 cm.

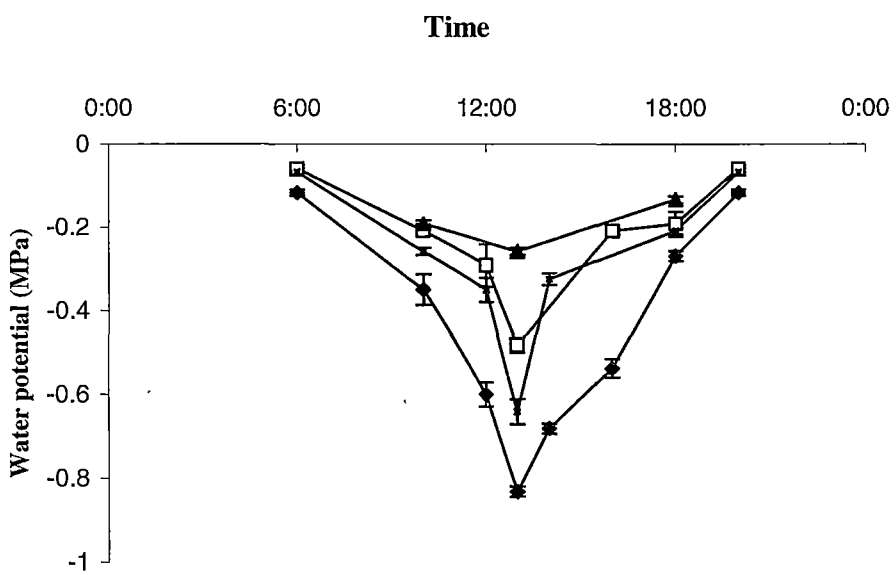


**Figure 20.** The time course of plant water potential in leaf (◆), stolon (■), tuber < 1.0 cm (□) and tuber > 2.0 cm (▲) in a sunny day in plants grown in November-December 2001. N = 4. Bars show SE.

## Leaf Water Potential

Environmental conditions affect the timing and absolute changes in leaf water potential. Significant differences ($p < 0.05$) in midday leaf water potential were recorded on sunny, cloudy and rainy days. The lowest midday leaf water potential was on sunny days, -0.84 ± 0.01 MPa, and the highest was on rainy day, -0.33 ± 0.03 MPa, while the midday leaf water potential on cloudy day was –0.54 ± 0.20 MPa (Table 16). However, when plants were grown under similar day conditions, the midday leaf water potential did not vary significantly. For example, leaf water potential measured at midday over three consecutive sunny days did not show significant differences (Table 16). The midday leaf water potential varied between -0.82 ± 0.08 to -0.92 ± 0.04 MPa. In order to minimise variation due to environmental conditions, all subsequent measurements of water potential were done on fine (cloud free) days.

**Table 16.** Leaf water potential related to weather conditions. Measurements were undertaken between 12:30 and 13:30 in crop grown in January-February 2002. Every figure is the mean of three measurements.

| | **Average PAR (12:00-14:00) ($\mu mol.m^{-2}.s^{-1}$)** | **Average temperature (12:00-14:00) (°C)** | **Midday leaf water potential (Average ± SE) (MPa)** |
|---|---|---|---|
| **Day 1 (sunny)** | 423 | 27.4 | -0.92 ± 0.04 |
| **Day 2 (sunny)** | 226 | 22.4 | -0.82 ± 0.08 |
| **Day 3 (sunny)** | 289 | 23.6 | -0.84 ± 0.01 |
| | | | |
| **Day 1 (sunny)** | 289 | 23.6 | -0.84 ± 0.01 |
| **Day 2 (Rainy)** | 164 | 23.0 | -0.33 ± 0.03 |
| **Day 3 (cloudy)** | 205 | 23.5 | -0.54 ± 0.20 |

The leaf water potentials measured at stage 2 (stolon elongation stage) in different growth seasons were shown in Fig. 21. The midday leaf water potential was lower

in November-December and January-February compared to that of May-June, July-August and September-October. The midday leaf water potential was -0.92 ± 0.02, -0.84 ± 0.02, -0.55 ± 0.03, -0.55 ± 0.03 and -0.53 ± 0.01 MPa for the January-February, November-December, September-October, July-August and May-June crops respectively. The timing of swelling (crop stage 2) for the November-December, September-October, July-August and May-June crops was 45, 27, 25 and 20 days after planting respectively, while the January-February crop did not develop any swelling tips at 47 days after planting when the trial was terminated. It appears that leaf water potential during the stolon elongation stage was lower in crops where tuber initiation was delayed.

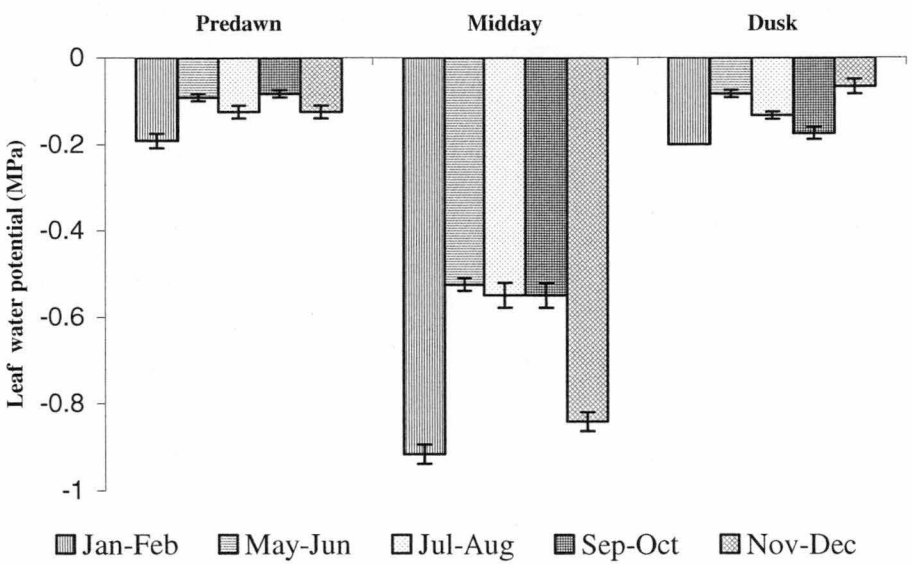


**Figure 21.** Leaf water potential measured in crops grown in January-February, May-June, July-August, September-October and November-December 2002. N=3. Bars show SE.

Leaf water potential at midday was significantly higher under SD compared to LD conditions ($p < 0.05$) (Fig. 22). Plants treated with paclobutrazol had significantly higher midday leaf water potential compared to paclobutrazol-untreated plants ($p < 0.05$) (Fig. 22). Leaf water potentials measured at predawn and dusk were similar for all treatments.

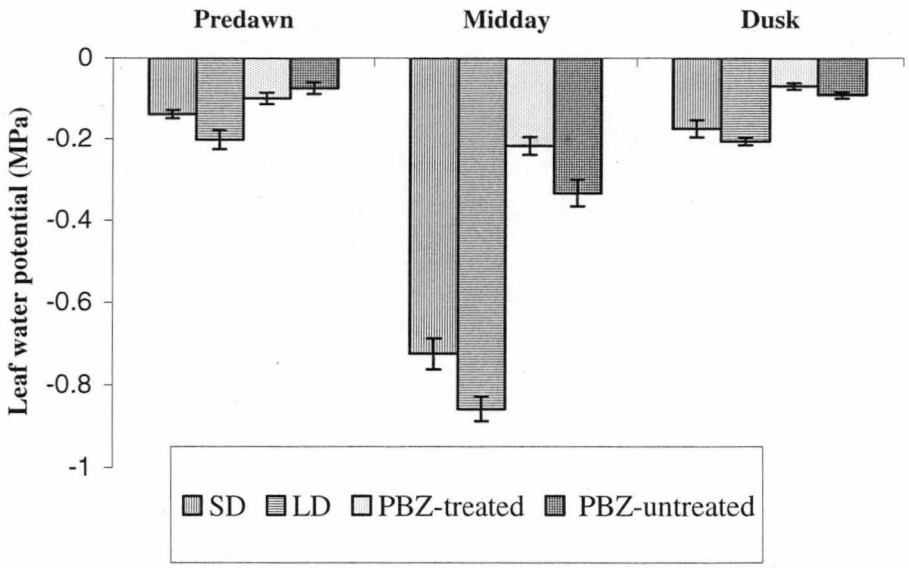


**Figure 22.** Leaf water potential measured 3 days after SD (8 hours, 10:00-18:00) and LD (14.5 hours, 6:00-20:30) treatment in plants grown in January-February 2002 and 3 days after treatment in paclobutrazol (PBZ)-treated and PBZ-untreated plants in the November-December 2001 crop. Measurement were taken at 5:00-6:00, 12:00-13:00 and 17:00-18:00 for "predawn", "midday" and "dusk" respectively. N = 3. Bars show SE.

## Stolon Water Status

Stolon water potential at predawn and dusk were similar but was significantly lower at midday (Fig. 23). This pattern was similar to the pattern of leaf water potential. Osmotic potential decreased from predawn to midday, and was maintained at approximately the same level until dusk. As water potential increased during the afternoon, the data suggest osmotic adjustment in the stolon during the day.

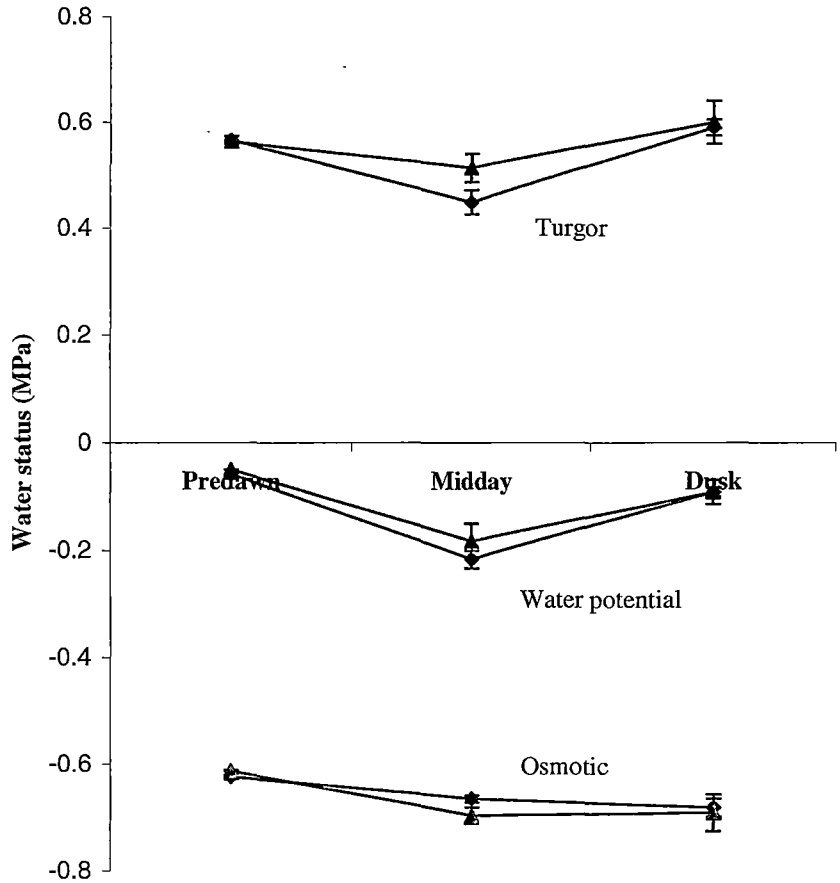


**Figure 23.** Stolon water potential, osmotic potential and turgor measured in sunny day in the January-February 2002 crop, where (♦) and (▲) stand for different days. N=3. Bars show SE.

Comparison of stolon water status in crops grown in different seasons revealed similar patterns to those of leaf water status (Fig. 24). The lowest stolon total water potential was found at midday, while total water potential was similar at predawn and dusk. Stolon osmotic potential decreased from predawn to midday and dusk. Stolon turgor decreased from predawn to midday but increased from midday to dusk.

Although the water potential in stolons was found to be similar in different growth seasons, the osmotic potential was significantly different ($p < 0.05$) between

seasons. This in turn led to a significant difference in stolon turgor ($p < 0.05$). As stolon osmotic potential was lower at dusk compared to that of predawn and the water potential was similar at predawn and dusk, maximum turgor potential was at dusk (Fig. 24).

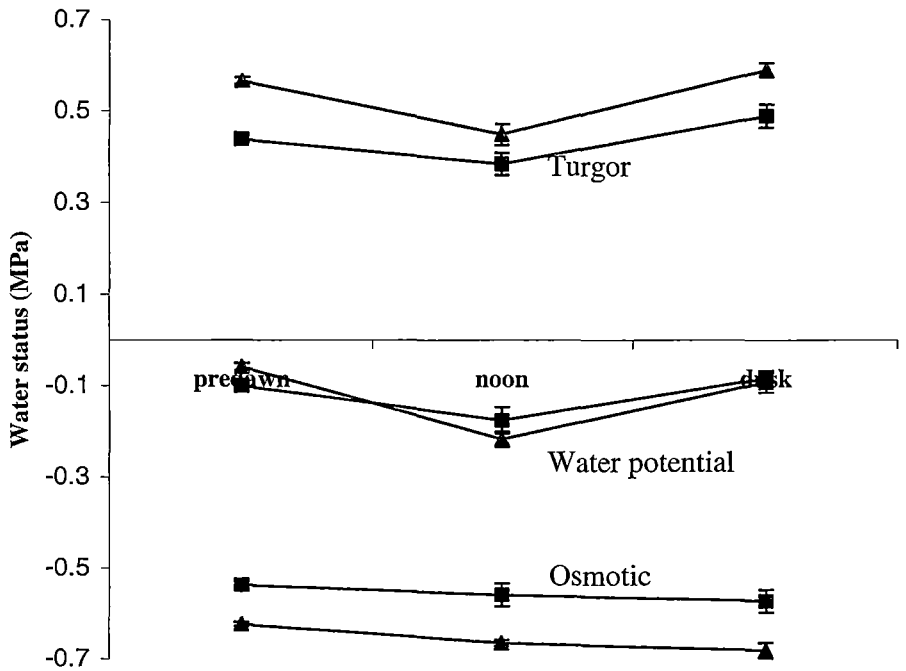


**Figure 24.** Stolon water potential, osmotic potential and turgor measured in a fine day in the January-February (▲) and November-December (■) crops 2002. N=3. Bars show SE.

The effect of day length on stolon water status was studied in the January-February 2002 crop. The main effect of daylength on stolon water status was found at midday (Fig. 25). Stolon total water potential at midday was higher under SD than LD ($p < 0.05$) but osmotic potential was lower ($p < 0.05$), which led to a significantly higher turgor in SD compared to LD plants ($p < 0.05$). No significant differences between day length treatments were found for stolon water potential, osmotic potential and turgor at predawn and dusk.

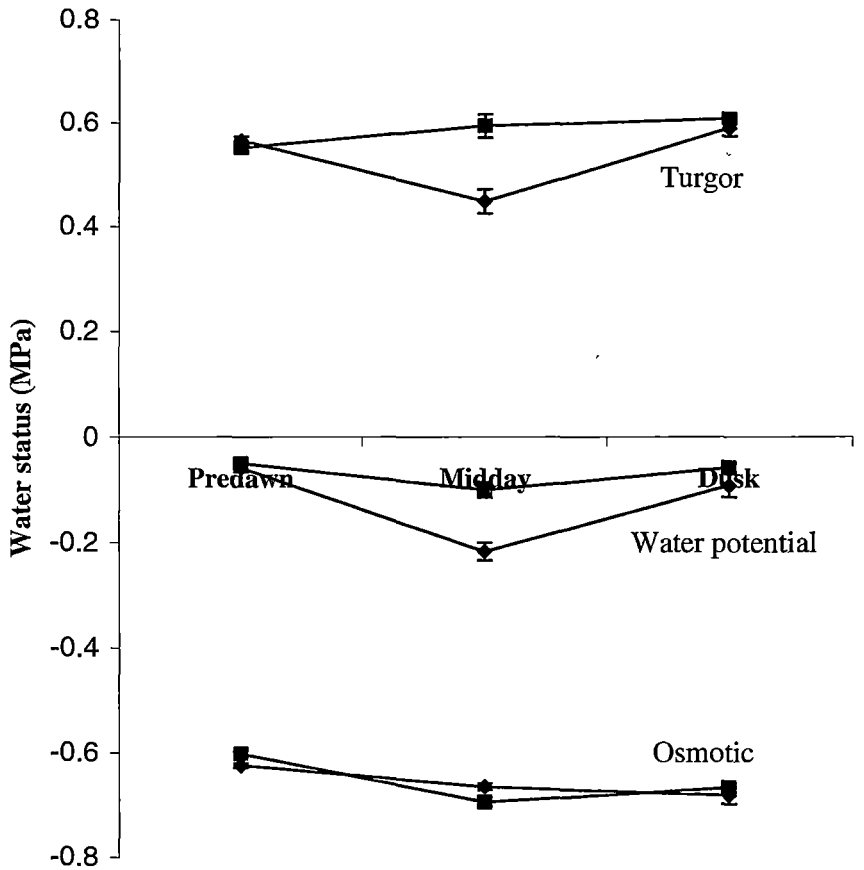


**Figure 25.** Stolon water potential, osmotic potential and turgor under SD (■) and LD (◆) treatments in the January-February 2002 crop. N=3. Bars show SE.

The effect of paclobutrazol on stolon water status (Fig. 26) was similar to that of SD treatment. The main effect of PBZ was at midday. PBZ decreased midday stolon water potential marginally ($p = 0.06$) but increased midday stolon turgor potential significantly ($p < 0.05$). PBZ did not affect stolon osmotic potential. Stolon water potential, osmotic potential and turgor at predawn and dusk were not significantly different between PBZ-untreated and PBZ-treated plants.

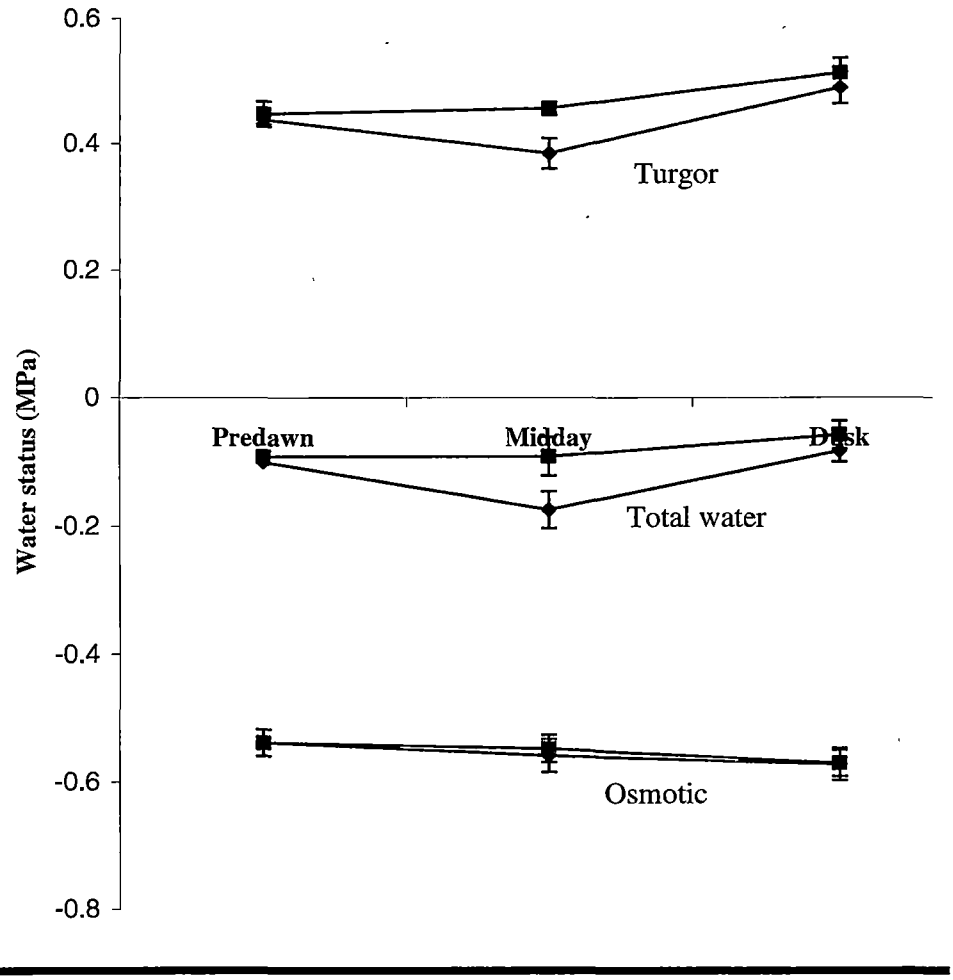


**Figure 26.** Stolon water potential, osmotic potential and turgor potential in PBZ-treated (■) and PBZ-untreated (◆) plants grown in November-December 2001. N=3. Bars show SE.

## Water Status in Stolons, Swelling Tips and Tubers

Significant differences in water status of stolons, swelling tips and tubers were recorded in samples collected from plants in the same crop on the same day (Fig. 27). Matched samples of the three organ types were collected from three separate plants, so the differences recorded were between organs rather than between plants. The water potential of stolons, swelling tips and tubers was similar at predawn and dusk. A significant difference between water potential was measured at midday.

Tuber water potential was higher compared to stolons and swelling tips ($p < 0.05$), while stolon and swelling tip had the similar water potential at midday.

Tuber osmotic potential measured at predawn, midday and dusk was generally lower than in swelling tips and swelling tips osmotic potential was generally lower than in stolons (Fig. 27). However, statistic analysis showed that the significant difference ($p < 0.05$) only existed between tubers and stolons measured at predawn and midday, and between swelling tips and stolons at midday.

There was a trend of increase in tissue turgor from stolons to swelling tips and to tubers (Fig. 27). Tuber turgor potential at predawn, midday and dusk was always higher than in stolons ($p < 0.05$). Tuber turgor was significantly higher than in swelling tips at midday ($p < 0.05$). Swelling tip turgor was not significantly higher than that of stolons.

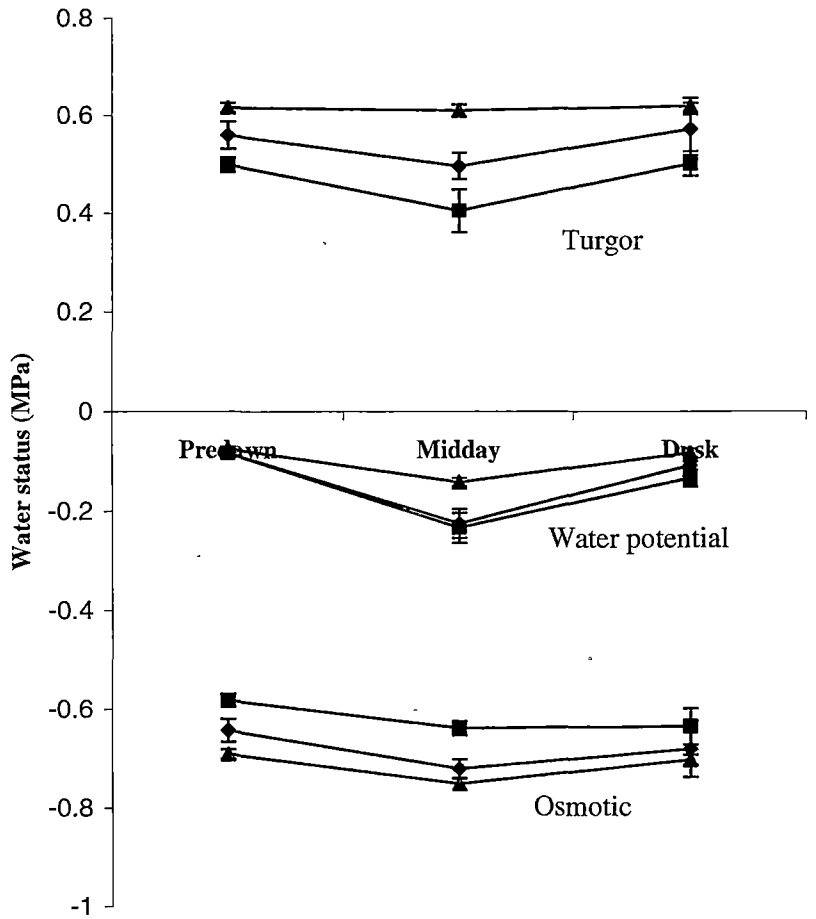


**Figure 27.** Water potential, osmotic potential and turgor in stolons (■), swelling tips (♦) and tubers (▲) measured at the same day in the September-October crop 2002. N=3. Bars show SE.

# DISCUSSION

The diurnal pattern of potato leaf water potential measured in plants grown in hydroponics was similar to that observed by Gandar and Tanner (1976). Leaf water potential decreased gradually from early morning to midday or early afternoon. After reaching the minimum value, water potential increased from late afternoon to nighttime. During the dark period, leaf water potential remained relatively constant. Daily fluctuations in leaf water potential varied with environmental factors. A lower minimum water potential was recorded in sunny compared to overcast conditions. Midday leaf water potential was lower in summer (December and February) than in autumn (May), winter (July) and spring (September). Midday leaf water potential in summer was in the range of reported by Gandar and Tanner (1976).

Midday leaf water potential showed a correlation with timing of tuber initiation. Early tuber initiation occurred in plants grown under condition with high midday leaf water potential and delayed tuber initiation from plants with low midday leaf water potential. This suggests that tuber initiation may be related to plant water status.

Stolon water potential has previously not been reported, probably due to the difficulty in obtaining suitable stolon material. The diurnal pattern of stolon water potential was found to be similar to that of leaf water potential, but the midday water potential in stolons was higher than that of the leaf. Similarly, tuber water potential followed the same diurnal pattern as leaves and stolons, but the minimum water potential at midday was higher than that of leaves and stolons. This gradient was identical to the report of Gandar and Tanner (1976). Transpiration from leaves decreased leaf water potential to produce a gradient in water potential from leaves to root, with the lower water potential in leaves and higher water potential in underground parts. Tuber water potential during the daytime was generally higher than that of stolons. Differences in hydraulic resistance in tubers and stolons may be an explanation for this result.

It has been concluded previously that daily changes in tuber water potential grown in dry soil were not closely related to changes in leaf water potential, but when grown in wet soil, tuber water potential was always higher than that in leaves (Gander and Tanner, 1976). In this study, tuber water potential was always higher than that of leaves. In addition, this study also showed that greater diurnal variation in leaf water potential was accompanied by greater variation in tuber and stolon water potential, which was similar to the results of Campbell (1972) and Campbell *et al.* (1976).

Stolon and tuber osmotic potential decreased from predawn to midday. This may be due to both water loss from and carbohydrate transported into these organs. However, from midday to dusk, osmotic potential remained relatively constant. Plants often tolerate water deficits by lowering cellular osmotic potentials (Turner and Jones, 1980). It is probably that from morning to midday when water potential decreased, accumulation of solutes occurred in stolons and tubers. This kind of response of plant to drought results in maintenance of a positive turgor potential, essential for many processes including growth (Meyer and Boyer, 1981). From midday to dusk when water potential increased, the high concentration of solutes resulted in water movement to these tissues, which led to high turgor potential. As a result, stolon and tuber turgor also undergo a diurnal rhythm. Turgor potential of stolons and tubers declined from predawn to midday but increased from midday to dusk.

The diurnal pattern of stolon and tuber growth (Chapter 5A) was similar to the pattern recorded for turgor potential. Turgor potential is important for plant growth because turgor is required to increase cell volume. The low stolon and tuber turgor at predawn corresponded to period of low growth rate, and high turgor at dusk was consistent with high growth rate.

High turgor observed in plants at dusk was mainly ascribed to the combination of the low osmotic potential and high total water potential. In a diurnal cycle, osmotic potential in stolons and tubers at midday were similar to that of dusk, however, the lowest total water potential decreased tissue turgor at midday. In contrast, the rapid increase in water potential led to rapid increase in tissue turgor at dusk. The low

osmotic potential in the afternoon was likely resulted from high carbohydrate concentration. Hofmann and Wunsch (1964) found that the concentration of sucrose followed a diurnal rhythm in stems, with highest values between 15:00 and 18:00 but the lowest values between 24:00 and sunrise.

The tuber inductive conditions, SD and PBZ, led to higher midday stolon turgor compared to that of control plants, suggesting a relationship between turgor and tuberization. High stolon turgor potential appeared to favour tuber initiation. The time of swelling initiation was shown to be from afternoon to early evening, which was the period of rapid growth for stolons and tubers (Chapter 5A) and also corresponded to high turgor potential at dusk. In addition, the concurrent measurement of water status in stolons, swelling tips and tubers revealed that a turgor potential gradient existed with tuber > swelling tip > stolon. This suggested that the process of tuber formation may be accompanied by a significant increase in tissue turgor.

# CHAPTER 6

# TURGOR AND TUBER INITIATION

## OUTLINE

***The measurement of stolon and tuber diurnal development patterns using the image capture and analysis method in Chapter 5A and of stolon and tuber water relations in Chapter 5B not only revealed a relationship between plant growth and turgor potential, but also suggested that turgor may play an important role in tuber initiation. This hypothesis was tested by investigating the correlation of turgor with stolon and tuber development, and tracing the change of turgor potential in stolon tips from elongation to expansion. The effect of turgor on tuber initiation was examined further by manipulating stolon turgor potential by altering nutrient solution osmotic potential.***

## INTRODUCTION

Stolon relative growth peaked a few days prior to visible stolon swelling when environmental factors were kept relatively constant, indicating a relationship between stolon elongation and tuber initiation (Chapter 3B). Investigation of stolon growth and tuber initiation using the image capture and analysis method identified that both rapid stolon elongation and tuber expansion occurred at the same period in a daily cycle, from afternoon to evening (Chapter 5A). This period corresponded to the timing of high tissue turgor (Chapter 5B). The commencement of swelling of the stolon tip occurred at the same period as the rapid stolon elongation and tuber expansion (Chapter 5B) implying that stolon tip swelling was related to turgor pressure. The diurnal measurement of plant water status from stolons, swelling tips and tubers indicated that turgor potential was

higher in tubers compared to swelling tips and in swelling tips compared to stolon (Chapter 5B). This suggested that stolon development from elongation to swelling and tuber expanding was accompanied by an increase in tissue turgor, and hence tissue turgor may play an important role during tuber formation.

This chapter investigated the relationships between turgor and the events of tuberization including stolon elongation, swelling and tuber expansion. The change in tissue turgor during the series of developmental events from stolon to tuber was measured, along with the corresponding changes in stolon elongation rate. The effect of turgor on stolon growth and tuber formation was tested by manipulating stolon turgor and measuring stolon elongation rate and timing of tuber initiation.

# LITERATURE REVIEW

## Plant Growth and Turgor Potential

Many previous studies have shown that the growth of plant species follows a diurnal growth pattern (Elfving and Kaufmann, 1972; Higgs and Jones, 1984; Tromp, 1984; Johnson *et al.*, 1992; Kitano *et al.*, 1996). The growth of potato tubers (Baker and Moorby, 1969; Stark and Halderson, 1987; Eguchi, 2000; Chapter 5A) and stolons (Chapter 5A) also follows a diurnal pattern. Rapid growth during the late afternoon to evening corresponds to increasing turgor (Chapter 5B), while from morning to midday growth decreased and turgor was at a minimum in the diurnal cycle.

Turgor pressure is generally considered as the driving force for cell expansion and cell division, hence plant growth (Dale, 1988; Findlay, 2001). Plants grow mostly by cell enlargement, although the continuous cell division in the meristem is prerequisite for plant growth. Cell division contributes less to plant growth in volume compared to cell expansion. Cell enlargement was sensitive to changes in tissue water potential (Hsiao, 1973; Volkmar and Woodbury, 1995). Cell

enlargement usually begins to slow down or stop at a water potential of only –0.2 to –0.4 MPa (Hsiao, 1973; Volkmar and Woodbury, 1995). Many other examples of reduction in cell enlargement and vegetative growth caused by water stress have been reported (e.g. Hsiao, 1973; Turner and Begg, 1978). However, cell division is considered less sensitive to water stress than cell elongation/expansion, hence turgor potential is less important in cell division in comparison to cell expansion (Acevedo *et al.*, 1971; Kleinendorst, 1975).

Expansion of plant cells is proportional to the difference between turgor potential and cell wall yielding stress (Lockhart, 1965), i.e.

Rate of growth = m (Turgor potential – Cell wall yielding stress)

The proportionality coefficient 'm' is referred to as cell wall extensibility. The 'cell wall yielding stress' and 'm' are thought to be the physical properties of the cell wall and may be modified by the metabolic processes of the cell (Boyer, 1987; Cleland, 1987; Ray, 1987). Growth in expanding tissue is therefore regulated by changes in water status (turgor) and cell wall properties.

The relationship between turgor potential and growth has been studied. Generally, reduction in turgor potential decreased plant growth (Robinson and Barritt, 1990). It was reported that 100 mmol/litre NaCl (which generates –0.45 MPa water potential at 25°C) completely inhibited microtuber development and decreased stolon growth in *Solanum tuberosum* (Silva *et al.*, 2001). A turgor reduction of 0.05 MPa in *Begonia* leaves by decreasing air relative humidity resulted in a 30 – 40 % reduction in the rate of elongation (Serpe and Matthews, 2000).

The physical properties of the cell wall may be modified by the metabolic processes of the cell (Boyer, 1987; Cleland, 1987; Ray, 1987) and the metabolic processes which control the extensibility and yielding stress may also be influenced by water stress and osmotic adjustment (Green *et al.*, 1971; Cleland, 1987).

Plant turgor changes are caused by the transport of solutes into or out of the cells. The transport of solutes builds up an osmotic gradient, which in turn causes a corresponding flow of water and a change in turgor of the cell. The main solutes in plant cell include organic molecules and potassium salts (Marschner, 1995; Talbott and Zeiger, 1998). For example, soluble carbohydrate is the main osmoticum in plant cell and the accumulation of hexoses (glucose and fructose) represents approximately 50 % of the total uptake of osmoticum by the growing region of *Helianthus annuus* (McNeil, 1976). Other studies reported that potassium concentrations contributed to plant cell osmotic potential, usually in the range of ¼ to ¾ of that due to sucrose in the sieve-tube sap's osmotic potential (Hoad and Peel, 1965; Mengel and Haeder, 1977; Smith and Milburn, 1980a, b). The accumulation of the solutes decreases osmotic potential that makes cells uptake water to achieve high turgor potential. Although there was no study of tissue turgor during tuber initiation, many literature reports provided evidence for a link between solutes and tuber initiation.

Early studies by Burt (1964) clearly showed a continuous increase in soluble sugars in stolon tips and leaves towards tuber initiation and tuber initiation was associated with a three- to four-fold increase in the concentration of soluble sugars (% dry wt) in the stolon tips and two-fold increase in leaves. While osmotic potential and turgor were not measured, it is likely that a change of this magnitude would have decreased osmotic potential and increased turgor potential. It was also reported that the soluble carbohydrate concentration in the stolon tips increased by about 25 % immediately after a period of low temperature treatment, and swellings of the stolon tips commenced at the end of the cold treatment (Burt, 1961). Other studies have also suggested that tuber initiation is associated with those conditions that lead to a high concentration of soluble carbohydrate at stolon tips. A continuous increase in soluble sugars (Renz *et al.*, 1993; Misra *et al.*, 1994; Ross *et al.*, 1994) and total carbohydrates, including sucrose, starch and reducing sugar (Hawker *et al.*, 1979) in stolon tips towards tuber formation has been documented. Increasing the level of sucrose in the stolon by antisensing the ADP-Glc pyrophosphorylase to prevent starch formation in the tubers led to an increased number of tubers (Muller-Rober *et al.*, 1992). As soluble carbohydrate

can represent a significant proportion of tissue osmoticum (McNeil, 1976), the increase in soluble carbohydrate is likely to have represented an increase in turgor.

## Manipulation of Plant Water Status in Hydroponics

Use of a hydroponic system for potato plant growth facilitated study of water status due to the ease in manipulating nutrient solution osmotic potential. It is possible to adjust nutrient solution osmotic potential by adding osmotically active materials such as polyethyleneglycol (PEG).

Adjusting nutrient solution osmotic potential in a hydroponic system in a relatively short time, for example a few hours, may change stolon and root water status without causing osmotic adjustment. Longer exposure to a reduced osmotic potential treatment resulted in osmotic adjustment (Bussis and Heineke, 1998). The term 'osmotic adjustment' has been applied to the increases in solute concentration in tissues undergoing a water deficit, where it can be shown that the changes arise from an increase in solutes and not from decreases in cell volume due to dehydration (Hsiao *et al.*, 1976; Turner and Jones, 1980).

Decreased nutrient solution osmotic potential by addition of polyethyleneglycol into solution has been demonstrated to induce osmotic adjustment in potatoes (Bussis and Heineke, 1998). Potato plants were able to grow when subjected to the water deficit induced by addition of 10 % (w/v) PEG 6000. Mannitol is another commonly used osmoticum and has been applied in *in vitro* study of aspects of potato tuberization. Mannitol solution has been used to investigate the effect of osmotic potential on micro-tuberization (Lo *et al.*, 1972; Garner, 1987; Chandra *et al.*, 1988). These authors found that media containing mannitol delayed or inhibited tuberization.

The manipulation of tissue turgor potential by exposing plants to water deficits can be imposed in hydroponics by the addition of osmotically active materials to the hydroponic solution. This method has been used to study osmotic adjustment in potatoes, but the effects of the water deficit treatment on stolon turgor, growth rate and timing of tuberization have not been examined.

# METHODS AND MATERIALS

The relationship between turgor and growth was studied in potato plants grown in August-September 2002. Potato plants for the experiment were grown under standard hydroponic conditions. A total number of 48 stolons and 15 tubers (diameter greater than 1.0 cm) were marked by placing a small cotton ball under each stolon at the late stolon elongation stage (stage 2) and tuber bulking stage (stage 5) respectively. Of the 48 stolons, 18 continued to elongate (stage 2), 24 reached the swelling tip stage (stage 3) and 6 progressed to tuber setting (stage 4). Daily measurements of length in the 48 stolons and diameter of the 15 tubers at tuber bulking stage (stage 5) were made prior to harvesting of samples for assessment of water status at approximately 17:00-18:00. Stolon length was measured using a ruler and tuber diameter was measured using a pair of callipers.

Six of the 48 stolons/swelling tips/small tubers and three of the 15 tubers with slow and rapid growth rate were harvested every day. Water potentials were measured using a pressure chamber (Chapter 5B). Plant samples were measured immediately after being excised. After measuring water potential, samples of stolons/swelling tips/small tubers and a cube of tuber tissue (5 x 5 x 5 $mm^3$) from the core of each large tuber were sealed in 1.5 ml centrifuged tubes and were snap frozen in liquid nitrogen and stored at –20 °C until required for analysis. Samples were thawed at room temperature for approximately 2 hours and the cell solution extracted by centrifugation at 12000 rpm for 5 minutes. The osmotic potential of the extracted solution was measured with a vapour pressure osmometer (Chapter 5B). Turgor was calculated by the difference between water potential and osmotic potential measured on the same sample.

It was often seen that swelling tips and non-swelling stolons occurred from branches of the same main stolon. In order to understand the water status in the branching swelling tips and stolons from the same main stolon, three of each of branched swelling tips and non-swelling tips from the same main stolon of the August-September 2002 plants were harvested at the same time at 18:00. Total

water potential and osmotic potential were measured and turgor was calculated as described above.

In order to test if stolon turgor influenced tuber initiation, tissue turgor was either increased or decreased by adjustment of nutrient solution osmotic potential. The experiment commenced in October 2002. When plants in every tray had formed more than 10 swelling tips (44 day after planting), treatments were applied to plants. Two out of six trays were used for control, two trays received distilled water in place of nutrient solution and two trays received PEG solution in place of nutrient solution. One tray of each treatment was used for destructive sampling and the second tray for assessment of growth rate and tuberization. 18 % PEG in nutrient solution (which decreases solution osmotic potential to -0.3 MPa) was supplied to the plant from 15:00 to 18:00. From 18:00 to 21:00 plants were then supplied with 14 % PEG solution (which decreases solution osmotic potential to -0.2 MPa). The distilled water treatment was commenced from 15:00 to 21:00. From 21:00 to 15:00, all plants were supplied with standard nutrient solution. The timing of application of the PEG and distilled water treatments was chosen to coincide with the period of rapid elongation of stolons. The aim of the treatments was therefore to increase (distilled water) or decrease (PEG) stolon turgor during the rapid elongation phase.

The use of PEG over mannitol in this study was suggested by Prof. Dr. Dieter Heineke (Personal communication), as contamination with mannitol consuming bacteria can lead to non reproducible growth conditions. In addition PEG was not detectable in leaves of plants grown in PEG solution for two weeks (Bussis and Heineke, 1998).

At the first day of treatment, at approximately 18:00, leaf and stolon water potential were measured from one tray of each treatment. Nine stolons from each of the PEG, distilled water and control treatments were labelled. Although 9 stolons from each treatment were selected, some of them developed necrotic lesions and died. Stolon length was measured daily at 21:00 from the day of treatment, and number of tubers and swelling tips per tray were counted. At day 7

of treatment, leaf water potential, stolon and swelling tip water potential, osmotic potential and turgor were measured at approximately 18:00.

Correlations between turgor potential and stolon/tuber development were analysed, and analysis of variance was carried out on turgor potential during tuberization (stolons to swelling tips and tubers) and the effect of experimental treatments on plant water status.

# RESULTS

## Turgor and Stolon Elongation

A positive relationship ($R^2 = 0.72$) was found between stolon relative elongation rate and turgor potential (Fig. 28). Stolon turgor measured at 18:00 ranged from 0.46 to 0.66 MPa. Stolon relative elongation rate varied from 0.04 to 0.35 cm/cm/day.

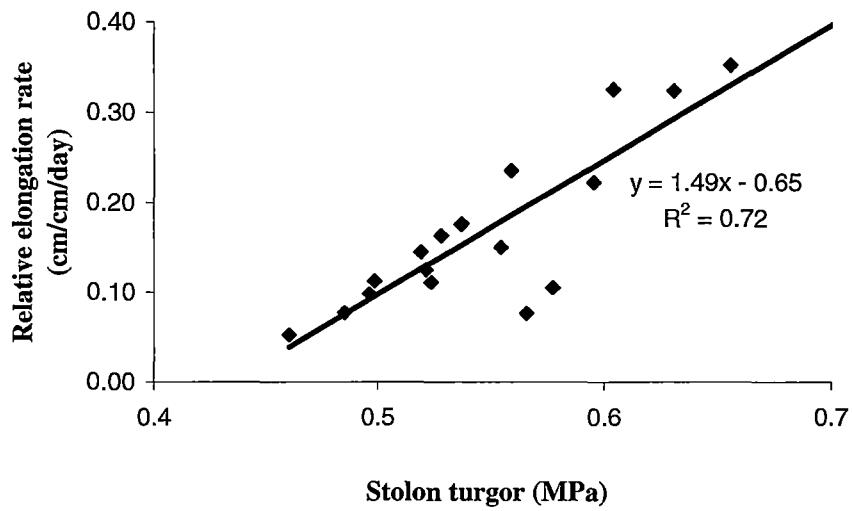


**Figure 28.** Stolon relative elongation rates against turgor potentials. Data were measured at 18:00 and collected over a period of 8 days from plants grown in August-September 2002.

## Turgor and Tuber Expansion

A positive relationship ($R^2 = 0.53$) was found between relative expansion rate and turgor potential of large tubers at stage 5 (tuber bulking stage) (Fig. 29). Turgor potential measured at 18:00 ranged from 0.54 to 0.67 MPa. Tuber relative expansion rate varied from 0 to 0.11 cm/cm/day.

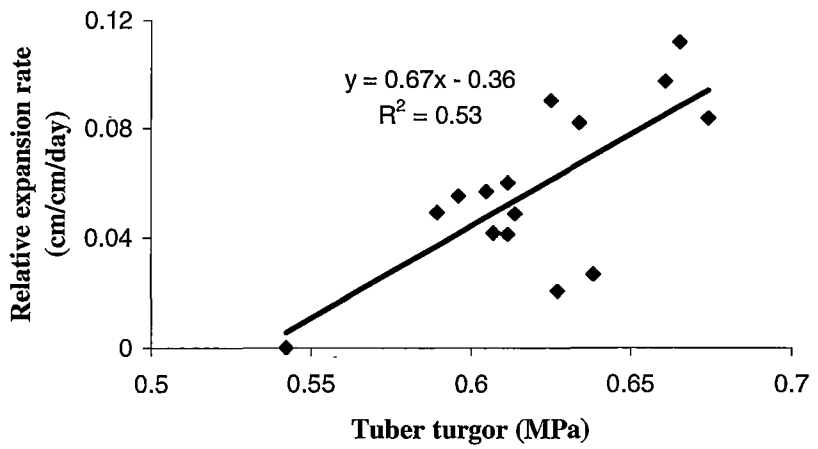


**Figure 29.** Tuber relative expansion rates against turgor potentials. Data were measured at 18:00 and collected over a period of 5 days at stage 5 from plants grown in August-September 2002.

## Turgor in Stolons, Swelling Tips and Mini-tubers

Turgor potential of stolons, swelling tips and tubers, measured during the rapid growth phase of the diurnal growth cycle at stage 2 (stolon elongation), stage 3 (swelling) and stage 4 (tuber setting) respectively, varied significantly (Fig. 30). Tissue turgor was marginally higher in tubers than that of swelling tips ($P = 0.07$), while turgor in swelling tips and tubers was significantly higher than that of non-swelling stolon tips ($P < 0.05$). Turgor potential in stolons, swelling tips and tubers was 0.55 ± 0.05, 0.65 ± 0.06 and 0.71 ± 0.08 MPa respectively. The ranges of values for stolons were 0.46 to 0.66 MPa, swelling tips were 0.55 to 0.78 MPa, and tubers were 0.60 to 0.83 MPa. Thus, while there was a trend of increasing turgor with development stage from stolons to tubers, there was not a discrete range of turgor values for each stage.

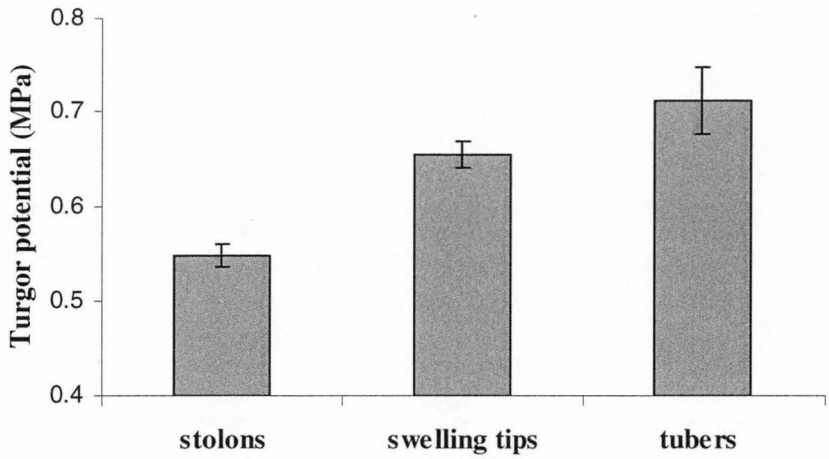


**Figure 30.** Turgor potential of stolons, swelling tips and tubers measured at 18:00 in different stages from plants grown in August-September 2002. Number of replicates was 18, 24 and 6 for stolons, swelling tips and tubers respectively. Bars show SE

## Turgor in Branching Stolons of the Same Main Stolon

Water status of stolons and swelling tips on branches growing from a single main stolon was measured. The total water potential in all stolon branches was the same but turgor in swelling tips was significant higher ($P < 0.05$) than that of non-swelling tips. The turgor was 0.60 ± 0.02 and 0.47 ± 0.01 MPa in swelling tips and non-swelling stolons respectively. The difference in tissue turgor was due to the significantly lower tissue osmotic potential in the swelling tips (Fig. 31).

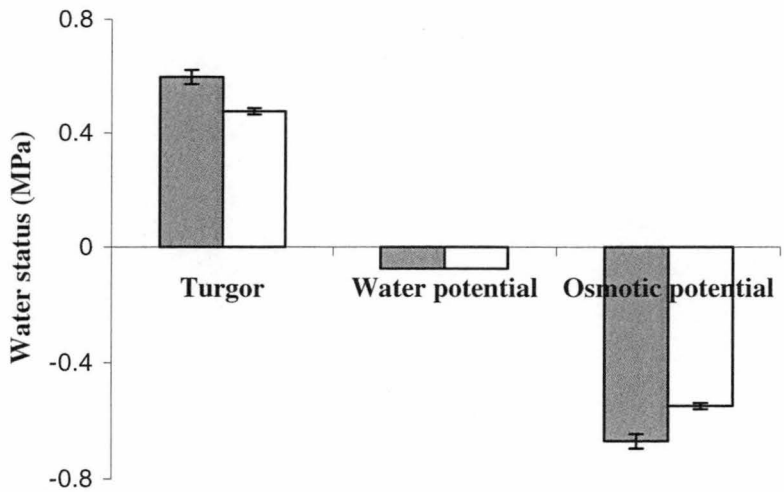


**Figure 31.** Water status measured from branching stolons (white column) and swelling tips (grey column) of the same main stolon at the time at 18:00 in August-September 2002. N=3. Bars show SE.

## Turgor During Tuberization

The gradient in turgor potential recorded in August-September 2002 was stolons < swelling tips < tubers (Fig. 32). The lowest tissue turgor was from stolon tips, with 77.8 % of samples less than 0.60 MPa, and the remaining 22.2 % between 0.60 and 0.66 MPa. Turgor of swelling tips varied between 0.55 and 0.78 MPa, with only 20.8 % of swelling tips having a turgor potential less than 0.6 MPa. The turgor potential of tubers varied from 0.60 to 0.83 MPa, with 66.7 % of samples greater than 0.70 MPa. The relative elongation rate was lower in tubers than in stolons and swelling tips, reflecting the cessation in stolon elongation after swelling had commenced. Elongation rate was generally lower in swelling stolons than non-swelling stolons, but 25.0 % of stolons at the swelling tip stage had relative elongation rates greater than 0.1 cm/cm/day, confirming the observation made in Chapter 3 that commencement of swelling may precede cessation of elongation.

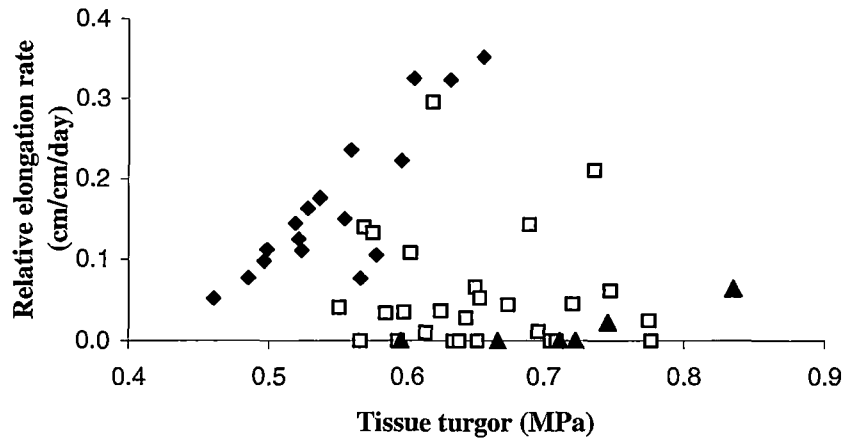


**Figure 32.** Turgor potential of stolons (◆), swelling tips (□) and tubers (▲) measured at 18:00 for a period of 8 days in plants grown in August-September 2002.

## Effect of Changes in Nutrient Solution Osmotic Potential

The effect of changing the hydroponic nutrient solution to either distilled water or nutrient solution plus PEG was assessed. On day one of the experiment, no significant difference in turgor potential was found between the PEG and control treatments, and between the distilled water and control treatments, while turgor in the distilled water treatment was significantly higher than in the PEG treatment ($p < 0.05$). Turgor potential in distilled water, PEG and control treatments were 0.56 ± 0.01, 0.53 ± 0.01 and 0.54 ± 0.03 MPa respectively (Fig. 33). Seven days after the treatments were imposed, turgor in the PEG treatment was significantly higher than in distilled water ($p < 0.05$), while turgor was not significantly different between PEG and control, and between distilled water and control treatments. Turgor in PEG, distilled water and control treatments was 0.59 ± 0.01, 0.55 ± 0.01 and 0.57 ± 0.003 MPa respectively. Turgor potential increased significantly from day one to day seven in the PEG treatment ($p < 0.05$), but did not change in the distilled water and control treatments.

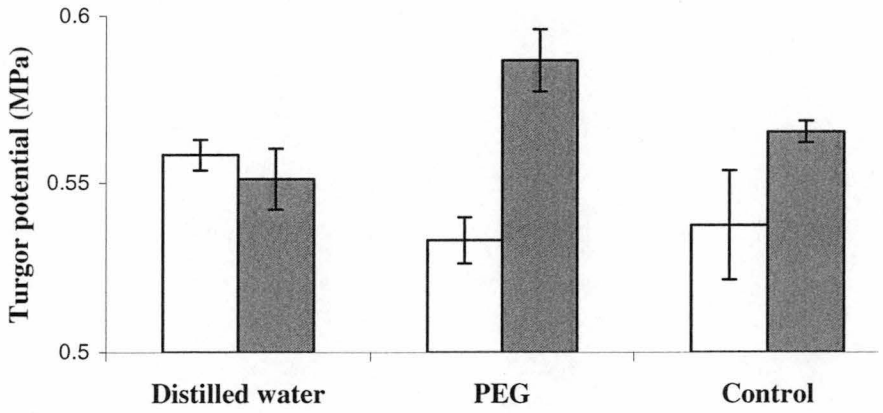


**Figure 33.** Stolon turgor potentials at day one (white column, N = 4) and day seven (grey column, N = 3) of treatments measured at 18:00 in the November-December crop (2002). Bars show SE.

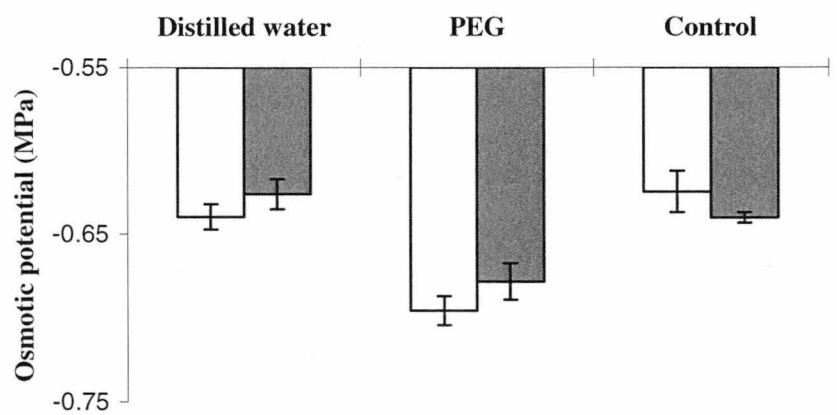


**Figure 34.** Stolon osmotic potentials of day one (white column, N = 4) and day seven (grey column, N = 3) of treatments measured at 18:00 in the November-December crop (2002). Bars show SE.

On day one of the experiment, PEG treatment decreased stolon osmotic potential significantly compared to the distilled water ($p < 0.05$) and control ($p < 0.05$) treatments, but no significant difference in osmotic potential was found between

the distilled water and control treatments. Osmotic potentials in the distilled water, PEG and control treatments were -0.64 ± 0.01, -0.70 ± 0.01 and -0.63 ± 0.01 respectively. On day seven of treatment, PEG resulted in significantly lower osmotic potential compared to the distilled water ($p < 0.05$) and control treatments ($p < 0.05$), but no significant difference in osmotic potential was found between the distilled water and control treatments. The osmotic potential in the distilled water, PEG and control treatments at day seven were –0.63 ± 0.01, -0.68 ± 0.01 and –0.64 ± 0.003 Mpa respectively. Osmotic potential did not show significant difference from day one to day seven in any of the treatments (Fig. 34).

Stolon relative elongation rate declined significantly between day one and day three in the PEG treatment and remained low until day five. A significant increase in growth rate was recorded in the PEG treatment between day five and day seven. While variations in relative growth rate from day to day were recorded in the control and distilled water treatments. The decline in growth rate between day two and day three corresponded to overcast weather conditions (Fig. 35).

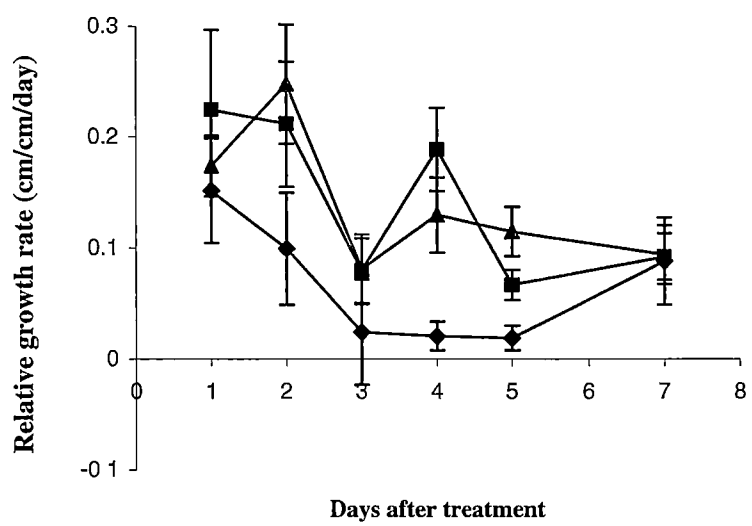


**Figure 35.** Stolon relative growth rate in PEG (◆), distilled water (▲) and control (■). Treatments lasted for 7 days. The number of replicates for PEG, distilled water and control was 6, 8 and 7 respectively. Days 2 and 3 were rainy days. Bars show SE.

The total number of swelling tips and tubers increased very slowly for four days after PEG treatment. However, from day 4 after treatment, total number increased rapidly in the PEG treatment (Fig. 36). Tuber initiation in the distilled water treatment was higher compared with the PEG and control treatments for 7 days.

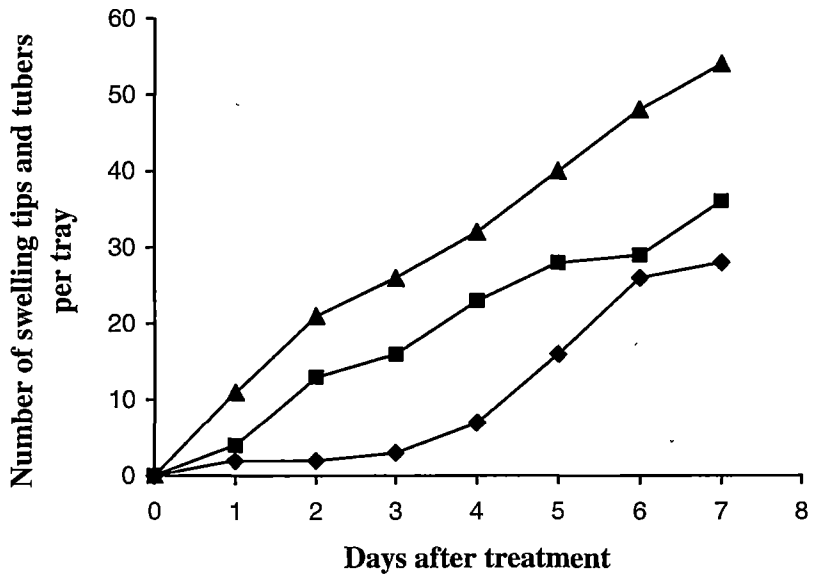


**Figure 36.** Total number of swelling tips and tubers after treatment of PEG (◆), distilled water (▲) and control (■).

# DISCUSSION

Strong positive relationships between tissue turgor and leaf growth have previously been documented for soybean grown in the field and growth chamber (Bunce, 1977). In this study, relationships between turgor and growth rate were described for stolons and tubers. Tissue turgor was correlated to stolon relative elongation rate ($R^2$ = 0.72). This suggested that high turgor leads to high stolon elongation rate and low turgor leads to low elongation rate. The relationship between tissue turgor and tuber expansion was weaker, with a coefficient of determination $R^2$ = 0.53. Tuber growth occurs both longitudinally and radially, and only radial expansion was measured in this study, so it is possible that a stronger relationship may exist between turgor and tuber volume.

A rapid stolon relative growth rate was shown to precede stolon swelling in hydroponically grown potatoes (Chapter 3). As rapid growth is accompanied by high turgor, this implies that a short time before swelling, an increase in stolon turgor must have occurred. Stolon relative growth rate declines as stolons progress through the swelling and tuber growth stages, while tuber expansion rate increases. Tissue turgor increased from elongating stolon to swelling tip and to newly-formed growing tuber, hence potato tuber formation was accompanied by increasing tissue turgor potential. When turgor was less than 0.55 MPa, stolons were never observed to swell, and all stolons with turgor higher than 0.66 MPa were recorded as swelling (Fig. 33). However, when stolon turgor was between 0.55 and 0.65 MPa, both elongating and swelling stolons were found (Fig. 33). It is therefore difficult to define critical turgor potentials for tuber initiation and it is likely that differences in tissue elasticity properties would need to be considered.

As plant tissue has to be cut for measuring total water potential and osmotic potential, it is impossible to trace the change of water status from stolon growth to tuber formation in the same stolon. However, elongating stolons and swelling tips can occur concurrently in branches of the same main stolon. This provides useful materials to compare the turgor pressure in elongating stolons and swelling tips as

the water potentials from the branches of the same main stolon at the same time are equal. The turgor of the branching stolons and swelling tips were shown to be different, where turgor of swelling tips was higher than that of stolons (Fig. 32). This further supports the viewpoint that the swelling of the stolon tip requires high tissue turgor.

The effect of tissue turgor on stolon elongation and tuberization was tested by controlling nutrient solution osmotic potential. The solution osmotic potential was adjusted by adding PEG into nutrient solution or replacing the nutrient solution with distilled water. It is clear that decreasing solution water potential by PEG at the rapid growth period decreased stolon turgor at the first day of treatment. Stolon relative growth rate decreased for five days following PEG treatment. Tuber number did not increase for the first 3 days, but tuber number increased rapidly from day 4 after PEG treatment.

Distilled water treatment increased stolon turgor water potential at day one of treatment, 0.56 ± 0.01 MPa. As described above in this chapter, at turgor potential of greater than 0.55 MPa, stolons may undergo swelling, hence distilled water treatment increased tuber number over the 7 day period of treatment. At the first day of PEG treatment, turgor potential decreased to 0.53 ± 0.01 MPa. This regime of low turgor in tissue may have lasted for 3 - 4 days, as tuberization was inhibited. However, by the end of the treatment a higher stolon turgor, 0.59 ± 0.01 MPa, was measured from the PEG treatment in comparison to distilled water and control treatments. Turgor in distilled water and control treatments were 0.55 ± 0.01 and 0.57 ± 0.003 MPa respectively. The highest level of turgor potential in PEG treatment corresponded to a rapid increase in total tuber number.

The change of tissue turgor from low at the first day of treatment to high at the end of the PEG treatment may reflect significant osmotic adjustment. When plants were subjected to water stress at the first day of treatment, stolon turgor potential declined. However, potato plants can accumulate osmotic active substances such as hexoses and amino acids in response to water deficit (Bussis and Heineke, 1998), this process was called osmoregulation or osmotic adjustment (Morgan, 1984). As a result of the osmotic adjustment, tissue turgor

was increased and growth was promoted when plants were supplied with normal nutrient solution

This indicated that adding PEG to decrease nutrient solution water potential could decrease tissue turgor if osmotic adjustment did not occur, and low tissue turgor decreased stolon elongation and inhibited tuber initiation. For the first few days, adding PEG to nutrient solution decreased stolon turgor, resulting in decreased stolon elongation and inhibition of swelling. When tissue turgor increased as osmotic adjustment occurred at the end of the treatment, stolon elongation and tuber initiation recovered. This supports the finding that high turgor promotes stolon elongation and swelling.

# CHAPTER 7

# GENERAL DISCUSSION

The physiology of potato tuberization has been the focus of many studies using *in vitro* and cutting techniques, while fewer studies based on whole plant systems have been published. As potato tuberization is a complex process involving the interaction of different plant organs and responses to external stimuli, the study of tuberization at the whole plant level can add insight to the knowledge gained from studies using isolated plant segments. The nutrient film technique (NFT) hydroponic system has been demonstrated in this project to be a valuable tool in whole plant research. This system permits free access to stolons and tubers to study growth patterns without destroying plant tissue, and easy access to tissue for destructive sampling in an investigation of important physiological changes occurring during tuberization in intact plants.

While NFT hydroponic systems have been used previously to study potato tuberization, the sequence and timing of morphological events during tuberization in hydroponics have been described in detail for the first time in this project and used to define key stages of development. The developmental events of potato tuberization in hydroponically grown plants in this research were mostly consistent with published descriptions of field- and pot-grown plants.

The overall morphology of stolons and tubers developed in this hydroponics was consistent with published papers, with stolons having elongated internodes and small scale leaves (Kumar and Wareing, 1972) and tubers having degenerated leaves and axillary buds, shortened internodes, and a radially expanded stem axis (Cutter, 1992). The first stolon developed in this hydroponics was at the most basal of node and later at higher nodes of the shoot system, which is similar to literature reports (Plaisted, 1957; Lovell and Booth, 1969; Cutter, 1992). The

appearance of leafy stolons (Booth, 1963; Struik *et al.*, 1989b) was also observed in this hydroponics orginating from higher nodes that were exposed to light. Stolon thickness varied greatly, similar to that reported by Helder *et al.* (1993a), from less than 1 mm to 8 mm in this study. The first swelling was from the subapical zone of the stolon, as described by Artschwager (1924), Plaisted (1957) and Vreugdenhil *et al.* (1999). The location of the early tuber initiation and position of large tubers, were found to be distributed across a range of stolon and stolon branch positions, which is comparable to the literature descriptions (Lovell and Booth, 1969; Wurr, 1977). The conclusion that no clear correlation between location of swelling stolon tips and branching order, or timing of stolon swelling and stolon branch age (Helder *et al.*, 1993a) was supported by this study in hydroponics. The formation of the characteristic hook stage during tuberization (Melchiorre *et al.*, 1997) was only observed under conditions where tuber initiation was significantly delayed in this research. At all other times swelling occurred uniformly around the stolon circumference, resulting in the stolon tip being aligned with the plane of stolon elongation. Previous papers have described the hooking of the stolon prior to swelling and the occurrence of swelling at the hooked region (Booth, 1963; Cutter, 1992; Melchiorre *et al.*, 1997). The appearance of tuber initials in *in vitro* systems appear similar to those recorded in hydroponics (see Fig. 1 and 2 in Xu *et al.*, 1998a), indicating that care should be taken in using the hook stage as an indicator of tuber initiation. Hooking of stolon tips was noted in this hydroponics, but was a characteristic feature of the diurnal cycle of stolon elongation rather than swelling. The tip of each stolon usually hooked around midday, during the phase of the slowest stolon elongation/tuber expansion, and was most pronounced under conditions where total daily stolon growth was low and tuber initiation was delayed. As stolon swelling occurred immediately after the period of slow elongation in the diurnal cycle, the likelihood of hooked stolons being present at swelling was increased under conditions where initiation was delayed.

Based on existing scales describing development of individual stolons (Vreugdenhil and Struik, 1989; Melchiorre *et al.*, 1997; Ulloa *et al.*, 1997) and the morphological descriptions in this study, five developmental stages from planting of tissue plantlets to tuber bulking were defined. The stages were plant

establishment, stolon elongation, swelling, tuber setting and tuber bulking stages. Each stage in this scale included a corresponding key event defining the transition between stages for individual stolons. Tuber initiation on all stolons within a plant was not synchronous, resulting in stolons at different stages being present on plants at any one time (Vreugdenhil and Struik, 1989; Helder *et al.*, 1993a). Stages at the plant and crop level therefore needed to be defined based on the proportions of stolons at specific stages. Development of the scale was critical to the project as it enabled the investigation of plant nutrient and water uptake at defined stages under different treatment conditions.

Nutrient uptake rate generally showed a trend of decline with plant age in this study. This is consistent with the conclusion of other studies that most nutrient element including N, P and K in potato leaves tended to decrease over the growing season (Lorenz and Tyler, 1983; Kunkel and Thorton, 1986). Changes in nutrient uptake were not specific to stages of stolon/tuber development. Although no direct relationships between nutrient uptake and stage of tuberization were found, altering nutrient availability has been shown to affect tuberization. While nutrients such as calcium have been shown to play a key role in tuberization (Balamani *et al.*, 1986), plants grown with an adequate supply of calcium do not appear to preferentially increase uptake of any specific nutrient at times of peak requirement. Nutrient elements can be accumulated and stored in the shoot system in the early developmental stages (Smith, 1968), and these elements can be translocated from shoot tissue when they are required during tuberization. Translocation of elements from shoots to tubers during tuber formation was reported by Hawkins (1946) and Vimala *et al.* (1990). As there is limited xylem flow into stolons (Nelson *et al.*, 1990) and tubers (Kratzke and Palta, 1985), the majority of the nutrient elements used for their growth must be transported from shoots via the phloem. Manipulation of tuberization by altering nutrient supply in hydroponics is therefore likely to be indirect, including either accumulation of nutrients in the shoot system, remobilisation of stored nutrients, or altering other physiological processes linked to tuber initiation.

Assessment of timing and rate of potato stolon and tuber development at 2-3 days intervals revealed that swelling commenced after the maximum stolon elongation

rate per plant had been achieved. The relationship between stolon elongation rate and timing of tuber initiation was at both the crop level and the individual stolon level. However, in a similar modified hydroponic culture system when the interval time between measurements was 5-7 days, Helder *et al.* (1993a) did not find this relationship. The frequency of observation in this study was unlikely to detect the relationship, as tuber initiation commences within one to five days of the peak in elongation rate.

Plant growth (Elfving and Kaufmann, 1972; Higgs and Jones, 1984; Tromp, 1984; Stark and Halderson, 1987; Johnson *et al.*, 1992; Kitano *et al.*, 1996; Eguchi, 2000) and plant water status (Slatyer, 1967; Gandar and Tanner, 1976) have been shown to change following characteristic diurnal patterns. Previous studies have shown that potato plants are especially sensitive to water stress (Gander, 1975; Campbell *et al.*, 1976) and that water stress is detrimental to tuberization. Plants that subjected to water stress before tuber initiation had fewer tubers (MacKerron and Jefferies, 1986; Haverkort *et al.*, 1990; Shock *et al.*, 1992). In this project, the relationships between potato plant water relations, diurnal growth patterns and tuberization have for the first time been examined in detail.

The diurnal pattern of potato tuber expansion in hydroponics was similar to previous descriptions (Stark and Halderson, 1987; Eguchi, 2000). The diurnal pattern of stolon growth was monitored for the first time in this project. Stolon elongation displayed a similar diurnal pattern as tuber expansion with a rapid growth from afternoon to night. The diurnal pattern of stolon/tuber growth was closely related to plant water relations. Turgor potential appeared to be the main factor regulating the diurnal growth, as the pattern of turgor change was very similar to the diurnal growth pattern, and turgor potential was strongly correlated with stolon elongation and tuber expansion rates. The turgor potential of the swelling tips peaked during the rapid growth period of stolons and tubers, from afternoon to early evening. This was consistent with the results of Bunce (1977) who found a linear relationship between elongation rate and turgor in leaves of field and growth chamber grown plants of soybean.

Tissue turgor increased during the processes from elongating stolons to swelling tips and tubers. Tuberization is a continuous growth process with a shift in the dominant place of growth from longitudinal during stolon elongation to radical during tuber expansion. Contrary to previous conclusions (Vreugdenhil and Struik, 1989) the cessation in longitudinal stolon growth does not precede stolon swelling. Increasing stolon turgor, at the time in the diurnal cycle when elongation rate is most rapid, accompanies an increase in stolon elongation rate and the transition to tuber development. Both turgor and tissue elasticity properties may influence tissue expansion and as the latter was not studied in this project, it was difficult to define critical turgor potentials for tuber initiation. However, stolons were never observed to swell when turgor was less than 0.55 MPa, and only swelling tips and tubers were formed with turgor higher than 0.66 MPa. Stolons either elongating or with swelling tips were found with turgor between 0.55 and 0.66 MPa.

Support for the causal link between turgor and swelling can be found in anatomical studies in the literature. During the initiation of radial tuber growth, cell enlargement in the subapical region precedes cell division (Sanz *et al.*, 1996; Vreugdenhil *et al.*, 1999). The increase in cell size is consistent with increasing turgor. Another of the earliest changes involved in tuber initiation is the reorientation of cortical microtubules (Fujino *et al.*, 1995). In elongating stolons, the microtubules are in longitudinal orientation, which limits cell development to the longitudinal direction. However, prior to tuber initiation, the microtubules are reorganized into a transverse orientation. Microtubule orientation has been demonstrated to be regulated by turgor pressure in Spirogyra cells (Iwate *et al.*, 2001), with low turgor favouring oblique or longitudinally microtubule orientation and higher turgor increasing the number of microtubules oriented transversely.

The earliest nutritional theory of tuber formation suggested that inductive environmental factors (eg short days) inhibited shoot growth, causing assimilate to accumulated in the stolon tips, leading to stolon swelling and tuber expansion (Wellensiek, 1929; Werner, 1934; Burt, 1964). Although more is now known about the regulatory systems of tuberization, components of the nutritional theory

may still be relevant. A reduction in plant shoot growth rate can lead to early tuberization, for example, withdrawal of nitrogen from a nutrient medium (Werner, 1934); decreased temperature (Burt, 1964) and stem girdling (Van Schreven, 1956). However, moving plants from long to short photoperiods did not cause a decrease in rate of leaf expansion until well after a strong tuber sink had been formed but promoted tuberization (Lorenzen and Ewing, 1990). Hence, reduction in shoot growth is not necessary for tuberization but reduction in shoot growth can promote tuberization.

Moving plants from LD to SD may have increased partitioning of carbohydrate to underground parts. Lorenzen and Ewing (1992) reported that the accumulation of starch in potato leaves during daytime was greater under LD than SD. However, during the night period, leaf starch concentration decreased rapidly in SD but did not change in LD conditions, showing more effective carbon partitioning to the underground parts under SD compared to that of LD or continuous lighting (Stutte *et al.*, 1996). The increased carbohydrate in underground parts, such as stolon, may have increased stolon turgor and promoted tuberization.

High concentrations of carbohydrates in stolon tips prior to and during tuberization have been reported (Slater, 1963; Ross *et al.*, 1994). Tuber initiation was associated with significant increase in the concentration of soluble sugars in the stolon tips and leaves (Burt, 1964; Hawker *et al.*, 1979; Misra *et al.*, 1994). The promotive effect of low temperature on tuberization was accompanied by a 25 percent increase in stolon carbohydrate over the control period immediately after the period of low temperature (Burt, 1964). Increasing the level of sucrose in the stolons by preventing starch formation by antisensing ADP-Glc pyrophosphory-lase led to an increased number of tubers both per plant and per stolon (Muller-Rober *et al.*, 1992). An increase in leaf starch accumulation can be detected after as few as 2 days in tuber inductive conditions (Jackson, 1999). This implies that tuber initiation and high concentration of sugar are associated, and supports the theory that higher turgor is part of the stimulus for tuberization.

The timing of initiation of swelling, at the time of rapid stolon/tuber growth, from afternoon to evening, coincided with the period in a daily cycle when sucrose

levels have been reported to peak. Hofmann and Wunsch (1964) reported that the concentration of sucrose followed a diurnal rhythm in stems of potato plants with the highest values between 15:00 and 18:00. Other studies revealed that the export of sucrose from leaves increased approximately 2 hours before the end of the light period (Li *et al.*, 1992).

Overall, the diurnal pattern of stolon elongation and tuber expansion corresponding to changes in turgor, the timing of swelling at the time of the rapid growth phase in a daily cycle and an increasing in turgor during the process of tuberization from elongating stolons to swelling tips and tubers, not only reveal that turgor is the driving force for stolon elongation and tuber expansion, but also suggests that turgor is part of tuberization stimulus. Although this study provided evidence of the important role of water relations in tuber initiation, the factors regulating turgor within stolons require further study. Examination of the changes induced by environmental variables such as daylength, along with identification of the components effecting the osmotic adjustment noted diurnally and under external treatments, will improve our understanding in this exciting new area of study in potato tuberization.

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